NEW ANALOGS OF ROSARAMICIN ISOLATED FROM A *MICROMONOSPORA* STRAIN

II. STRUCTURE DETERMINATION

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(Received for publication December 25, 1989)

New 16-membered macrolide antibiotics 6108 A_1 (**1**), B (**2**), C (**3**) and D (**4**) were isolated from the culture filtrate of a *Micromonospora fastidiosus* sp.[1]. From the physico-chemical, spectroscopic data and chemical degradation studies, these compounds were shown to be analogs of rosaramicin (**5**)[2], whose structures were different from **5** at the C-18 position. In this paper, we describe the structure elucidation of these compounds.

The physico-chemical data of **1**~**4** are summarized in the previous paper[1]. The molecular formula of **1** was determined as $C_{33}H_{55}N_3O_9$ by HRFAB-MS (Calcd: *m/z* 638.4017, Found: *m/z* 638.4043 $(M+H)^+$). The IR spectrum (KBr) of **1** was similar to that of rosaramicin, but showed also a characteristic absorption at 1680 cm^{-1}. The ^1H NMR spectrum of **1** showed two sets of signals in the down-field region (Table 1). The ratio of these signals varied with solvent and temperature and the data suggested that **1** is a mixture of isomers. On treatment with dil-HCl, **1** afforded rosaramicin quantitatively. The lack of the aldehyde proton in the ^1H NMR of **1** and the chemical degradation result, suggested that **1** should be an analog of **5** substituted at the C-18 position. By a comparison of the ^{13}C NMR data (Table 2) between **1** and **5**, it was found that the signal of the aldehyde carbon (δ 202.7) in **5** was missing and signals at δ 168.6 (s), 152.6 (d) and 20.9 (q) were newly observed in **1**. These data suggested one of two possible partial structures for **1** as $-CH=N-NH-C(O)-CH_3$ (A) or $-CH=N-C(O)-NH-CH_3$ (B).

The ^1H (δ 1.95) and ^{13}C (δ 20.9) chemical shifts of the methyl group and its appearance as a singlet in the ^1H NMR spectrum in $CDCl_3$, support the structure of (A) which also accounts for the existence of *syn* and *anti* isomeric forms about the hydrazino double bond. In order to confirm it, **5** was treated with acetohydrazide at pH 4 to afford **1**. From these results, the structure of **1** was determined as shown in Fig. 1.

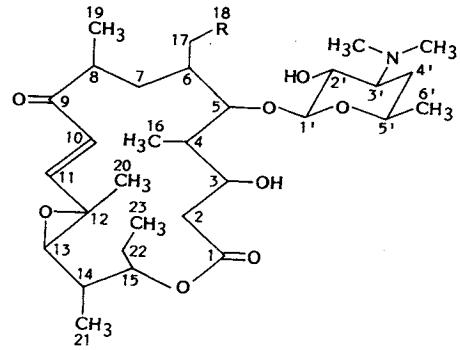
The MW of **2** was determined as 597 by FAB-MS: *m/z* 598 $(M+H)^+$. The ^1H and ^{13}C NMR data of **2** are shown in Tables 1 and 2, respectively. Chemical shift assignments for **2** were determined from ^1H-^1H COSY and ^{13}C-^1H COSY spectra of **2** and comparison with those of **5**†. The ^1H and ^{13}C NMR spectra of **2** were quite similar to those of **5** except that an aldehyde carbon at δ 202.7 in **5** was not observed in **2**, and instead a carboxylate carbon was found at 179.1 ppm. The presence of a carboxylate group was confirmed by the IR spectrum (KBr) cm^{-1} 1560 and 1385. These data suggested the structure **2** as shown in Fig. 1.

The MW of **3** was determined as 702 by FAB-MS (*m/z* 703 $(M+H)^+$, cesium iodide spikes *m/z* 835 $(M+Cs)^+$). The molecular formula of **3** was established to be $C_{34}H_{58}N_2O_{11}S$ from the MW and the results of elemental analysis (Calcd: C 58.10, H 8.32, N 3.99, S 4.56; Found: C 58.02, H 8.28, N 3.95, S 5.19.) The IR spectrum (KBr) of **3** showed the absorption of carboxylate (1615 and 1400 cm^{-1}). The ^1H and ^{13}C NMR data of **3** are listed in Tables 1 and 2, respectively. Comparison of these data with those of **5** indicated additional signals at δ_H 3.56 (dd), 3.23 (dd) and 2.85 (dd), coupled to each other in the ^1H NMR spectrum and at δ_c 38.8 (t), 69.7 (d) and 177.0 (s) in the ^{13}C spectrum. From these data, the presence of a cysteine moiety was suggested. In fact, the hydrolysis of **3** (pH 5, room temperature, 10 days) gave **5** and cysteine. These data and the chemical shift of C-18 (δ_H 4.53, δ_C 67.6), revealed that the sulfur atom in cysteine was connected to C-18. In order to confirm it, **5** was condensed with cysteine to give the cysteine adduct which was identical in all respects to **3** (^1H NMR, FAB-MS). It is known that macrolides having an aldehyde group, condense with cysteine to afford thiazolidine derivatives upon dehydration[5]. However, the FAB-MS and NMR data clearly establish the structure of **3** in the open chain form, as shown in Fig. 1, although **3** was easily dehydrated to yield the thiazolidine derivative.

The MW of **4** was determined as 621 by FAB-MS (*m/z* 622 $(M+H)^+$). The IR spectrum of **4** was similar to that of **5** with the exception of the

† Some of the ^{13}C NMR assignments of rosaramicin, published earlier[3,4], proved to be incorrect from our ^1H-^1H COSY and ^{13}C-^1H COSY connectivity measurements.

Fig. 1. Structures of 6018 A_1 (**1**), B (**2**), C (**3**), D (**4**) and rosaramicin (**5**).



6108 A_1 (**1**) $R = \overset{18}{C}H{=}N{-}NH{-}\overset{24}{C}O{-}\overset{25}{C}H_3$

6108 B (**2**) $R = \overset{18}{C}OOH$

6108 C (**3**) $R = \overset{18}{C}H(OH){-}S{-}\overset{1''}{C}H_2{-}\overset{2''}{C}H(NH_2){-}\overset{3''}{C}OOH$

6108 D (**4**) $R = \overset{18}{C}H{=}\overset{1''}{C}H{-}\overset{2''}{C}O{-}\overset{3''}{C}H_3$

Rosaramicin (**5**) $R = \overset{18}{C}HO$

Table 1. ^1H NMR data of 6108 A_1 (**1**), B (**2**), C (**3**), D (**4**) and rosaramicin (**5**).

Proton	**1** (major) - **1′** (minor)[a] (0.8 : 0.2)		**2**[b]	**3**[a]	**4**[b]	**5**[b]
18	7.64 (t)	7.35 (t)	—	4.53 (dd)	6.82 (ddd)	9.73 (s)
11	6.70 (d)	6.73 (d)	6.50 (d)	6.67 (d)	6.54 (d)	6.53 (d)
10	6.42 (d)	6.42 (d)	6.45 (d)	6.43 (d)	6.43 (d)	6.46 (d)
15	4.83 (m)	4.83 (m)	4.81 (m)	4.82 (m)	4.88 (m)	4.89 (m)
1′	4.18 (d)	4.25 (d)	4.28 (d)	4.37 (d)	4.28 (d)	4.22 (d)
3	4.02 (br d)	3.79 (br d)	4.01 (br d)	3.79 (br d)	3.80 (br d)	3.91 (br d)
5	3.79 (br d)	3.68 (br d)	3.63 (br d)	3.68 (br d)	3.65 (br d)	3.71 (br d)
5′	3.42 ~ 3.56 (m)		3.60 (m)	3.63 (m)	3.51 (m)	3.46 (m)
2′	3.27 (dd)	3.27 (dd)	3.31 (dd)	3.35 ~ 3.45 (m)[c]	3.22 (dd)	3.20 (dd)
3′	[c]	[c]	2.98 (m)	3.35 ~ 3.45 (m)[c]	2.50 (m)	2.49 (m)
13	[c]	[c]	2.83 (d)	2.85 (d)	2.80 (d)	2.82 (d)
17	[c]	[c]	2.77 (m)	2.41 (m)	2.71 (m)	3.08 (m)
	[c]	[c]	2.36 (m)	~1.70 (m)	2.38 (dd)	2.46 (m)
2	[c]	[c]	2.63 (dd)	2.60 (dd)	2.61 (dd)	2.65 (dd)
	[c]	[c]	2.13 (d)	2.20 (d)	2.09 (d)	2.10 (d)
8	[c]	[c]	2.60 (m)	2.70 (m)	2.69 (m)	2.55 (m)
N-CH_3	2.38 (s)	2.38 (s)	2.50 (s)	2.81 (s)	2.29 (s)	2.29 (s)
25	1.95 (s)	2.19 (s)				
2″				3.56 (dd)		
1″				3.23 (dd)		
				2.85 (dd)		
1‴					6.11 (d)	
3‴					2.24 (s)	

[a] In CD_3OD.
[b] In $CDCl_3$.
[c] Overlapping resonances.

absorption at 1670 cm^{-1} (α,β-unsaturated carbonyl). The ^1H NMR spectrum of **4** showed two olefinic protons at δ 6.11 (d, J=16.1 Hz) and δ 6.82 (ddd, J=6.2, 7.0 and 16.1 Hz) and a methyl proton at δ 2.24 (s) in addition to the protons of **5**, except for the aldehyde proton (Table 1). Comparison of the ^1H-^1H COSY and ^{13}C-^1H COSY data between **4** and **5**, suggested that **4** had an α,β-unsaturated ketone moiety. The partial structure was confirmed from the results of long range selective proton decoupling (LSPD) experiments, which demonstrated that the signals at δ 26.5 and δ 198.8 collapsed by irradiation of the olefinic proton at δ_H 6.11. The geometry of the double bond was shown to have the *E* configuration from the vicinal coupling constant of J=16.1 Hz. Other NMR data of **4**, except for the α,β-unsatulated ketone moiety, were quite similar to those of **5**, supporting the structure of **4** as shown in Fig. 1. This was confirmed by synthesis by treatment of **5** with triphenylphosphoranylidene-2-propanone in aqueous methanol (pH 7.5) at 60°C for 4 hours to give a product

Table 2. ^{13}C NMR data of 6108 A_1 (**1**), B (**2**), C (**3**), D (**4**) and rosaramicin (**5**).

Carbon	**1** (major)	**1'** (minor)[a]	**2**[b]	**3**[a]	**4**[b]	**5**[b]	**5**[f]
1	173.2 (s)	174.0	172.4	173.0	173.7	173.1	173.5
2	33.0 (t)[c]	*	40.1	40.7	39.5	39.6	39.7
3	66.9 (d)	67.6	66.9	66.6	66.9	66.6	68.0
4	42.9 (d)	42.1	42.7	42.4	40.9	41.1	45.1
5	80.8 (d)	82.0	83.4	80.4	81.5	81.0	81.3
6	34.9 (d)[d]	36.2[e]	33.4	35.8	36.0	31.1	31.4
7	32.9 (t)[c]	*	32.1	31.4	32.7	31.7	31.8
8	46.4 (d)	46.4	45.2	46.4	45.1	45.0	37.9
9	203.1 (s)	203.0	200.9	202.4	200.6	200.0	200.9
10	124.0 (d)	124.3	122.7	124.3	122.6	122.5	122.8
11	152.1 (d)	150.1	150.3	151.8	150.5	150.6	150.9
12	60.9 (s)	60.9	59.7	61.0	59.6	59.6	59.7
13	69.4 (d)	69.2	68.4	69.6	67.8	67.8	66.8
14	39.1 (d)[d]	38.9[e]	37.7	39.0	37.8	37.8	41.3
15	77.4 (d)	77.7	76.2	77.5	76.7	76.6	76.8
16	9.5 (q)	9.4	8.9	10.1	9.1	8.9	9.0
17	41.3 (t)	40.7	35.4	36.5	32.2	43.7	43.8
18	152.6 (d)	151.8 (d)	179.1 (s)	67.6 (d)	148.7 (d)	202.7 (d)	202.9
19	17.6 (q)	17.6	17.5	17.8	17.4	17.3	17.4
20	15.1 (q)	15.2	14.8	15.4	14.9	14.9	15.0
21	14.6 (q)	14.6	14.5	14.8	14.5	14.5	14.5
22	25.5 (t)	25.5	24.5	25.5	24.7	24.6	24.7
23	9.5 (q)	9.7	8.9	9.7	9.1	9.0	9.1
1'	104.9 (d)	105.4	104.3	104.5	104.4	104.1	104.5
2'	71.8 (d)	71.8	70.5	70.3	70.3	70.2	70.4
3'	65.6 (d)	65.7	65.3	66.3	65.6	65.6	65.8
4'	31.6 (t)[a]	*	29.6	33.5	28.3	28.4	28.4
5'	69.7 (d)	69.9	68.8	69.5	69.4	69.5	69.7
6'	21.4 (q)	21.4	21.0	22.0	21.3	21.0	21.2
N-CH_3	40.6 (q)	40.6	39.7	39.9	40.1	40.2	40.5
24	168.6 (s)	168.6					
25	20.9 (q)	20.3					
1''				38.8 (t)			
2''				69.7 (d)			
3''				177.0 (s)			
1'''					132.1 (d)		
2'''					198.8 (s)		
3'''					26.5 (q)		

[a] In CD_3OD.
[b] In $CDCl_3$.
[c~e] Assignments may be interchanged.
[f] Assignments in $CDCl_3$ in ref 4.
* Unclear.

identical in all respects with **4**.

In conclusion, we have elucidated the structures of four new macrolides, 6108 A_1, B, C and D by spectroscopic comparison with rosaramicin and chemical derivation from rosaramicin.

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