The Structures of Sulfomycins II and III

JUN KOHNO, NORIAKI KAMEDA, MAKI NISHIO*, AKIO KINUMAKI† and SABURO KOMATSUBARA

Lead Generation Research Laboratory at Toda and
† Lead Optimization Research Laboratory,
Tanabe Seiyaku Co., Ltd.,
2-2-50, Kawagishi, Toda-shi, Saitama 335, Japan

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The isolation and biological activities of sulfur-containing antibiotics sulfomycins I, II and III from the fermentation broth of *Streptomyces viridochromogenes* MCRL-0368 were reported by EGAWA *et al.* in 1969.[1] The structure of sulfomycin I was determined on the basis of chemical degradations and NMR techniques[2,3] to be a cyclic thiopeptide antibiotic represented by thiostrepton[4], berninamycin[3] and thiopeptin[5]. The structures of sulfomycins II and III, however, remained unclear. Recently, SETO and his colleagues reported a series of new thiopeptide antibiotics, promothiocins A and B[6], geninthiocin[7], thiotipin[8] and promoinducin[9], as *tip A* promoter-inducing substances. In this paper, we wish to disclose the structures of sulfomycins II and III based on the 1D and 2D NMR studies.

Purification of sulfomycins II and III from the crude extract[1] was achieved by successive column chromatographies on silica gel, reverse-phase ODS and preparative HPLC. The molecular formulae of sulfomycins II and III were determined to be $C_{54}H_{52}N_{16}O_{15}S_2$ and $C_{53}H_{50}N_{16}O_{16}S_2$, respectively, by HRFAB-MS or FAB-MS (sulfomycin II; *m/z* found 1251.3130, calcd 1251.3140 for $C_{54}H_{52}N_{16}O_{15}S_2Na$, sulfomycin III; *m/z* 1253 $(M+Na)^+$) and NMR data. The ^1H and ^{13}C NMR data of sulfomycins II and III are shown in Table 1.

The ^{13}C NMR spectrum of sulfomycin II displayed 54 signals composed of CH_3–C × 5, CH_3–O × 1, –CH_2– × 1, >CH– × 3, –CH_2= × 5, –CH= × 8, >C= × 21 and carbonyl C × 10. Although the DQF-COSY data revealed only the presence of three partial structures (–CH=CH–, CH_3–CH(OH)–CH–NH– and CH_3–

Table 1. ^1H and ^{13}C NMR data of sulfomycins II and III.[a]

Position	^{13}C (δ)		^1H (δ)	
	II	III	II	III
Thiazole (1)				
2-C	162.5	162.5		
4-C	149.0	148.9		
5-CH	127.2	127.1	8.54 (s)	8.53 (s)
CO	160.6	160.6		
Threonine				
NH			8.17 (d, 8.1)[b]	8.14 (d, 8.1)
αCH	59.2	59.2	4.36 (dd, 8.3, 4.6)	4.32 (dd, 8.3, 4.0)
βCH	66.2	66.1	4.18 (m)	4.17 (m)
γCH_3	20.1	20.1	1.10 (d, 6.9)	1.08 (d, 6.4)
OH			5.18 (d, 5.7)	5.20 (d, 5.7)
CO	169.1	169.0		
Oxazole (1)				
NH			9.31 (s)	9.37 (s)
αC	121.9	120.9		
βCH	134.2	131.6	6.37 (t, 7.5)	6.35 (t, 6.0)
γCH_2/CH	20.8	57.7	2.18 (m)	4.07 (m)
δCH_3	12.7		1.01 (t, 7.5)	
OH				4.92 (t, 5.7)
2-C	156.7	156.2		
4-C	128.6	128.7		
5-C	153.4	153.4		
5-CH_3	11.4	11.4	2.57 (s)	2.59(s)
CO	161.3	161.2		
Thiazole (2)				
NH			8.39 (d, 9.5)	8.41 (d, 9.7)
αCH	77.3	77.3	6.47 (d, 9.7)	6.47 (d, 9.5)
OCH_3	55.3	55.3	3.27 (s)	3.28 (s)
2-C	167.5	167.5		
4-C	148.9	148.9		
5-CH	126.7	126.7	8.45 (s)	8.47 (s)
CO	159.1	159.0		
Oxazole (2)				
NH			10.00 (s)	9.98 (s)
αCH	123.5	123.5		
βCH	129.3	129.4	6.52 (q, 7.3)	6.53 (q, 7.1)
γCH_3	13.6	13.6	1.78 (d, 7.5)	1.77 (d, 7.0)
2-C	156.8	156.8		
4-C	129.2	129.2		
5-C	153.7	154.1		
5-CH_3	11.4	11.4	2.58 (s)	2.59 (s)
CO	159.8	159.7		
Dehydroalanine (1)				
NH			9.19 (s)	9.17 (s)
αC	133.6	133.5		
βCH_2	104.9	104.7	5.71 (s), 6.41 (s)	5.70 (s), 6.42 (s)
CO	162.6	162.6		
Oxazole (3)				
NH			9.90 (s)	9.88 (s)
αC	129.5	129.5		
βCH_2	112.1	112.1	5.79 (s), 5.69 (s)	5.79 (s), 5.69 (s)
2-C	158.2	158.2		
4-C	138.9	138.9		
5-CH	140.1	140.1	8.65 (s)	8.65 (s)
Pyridine				
2-C	149.1	149.1		
3-C	130.7	130.6		
4-CH	140.2	140.2	8.70 (d, 8.1)	8.68 (d, 8.2)
5-CH	121.7	121.7	8.30 (d, 8.1)	8.30 (d, 8.2)
6-C	146.7	146.8		
CO	161.4	161.4		
Dehydroalanine (2)				
NH			10.43 (s)	10.43 (s)
αC	134.0	134.0		
βCH_2	105.6	105.7	5.96 (s), 6.59 (s)	5.96 (s), 6.58 (s)
CO	162.9	162.9		
Dehydroalanine (3)				
NH			10.07 (s)	9.98 (s)
αC	136.8	136.8		
βCH_2	111.1	111.2	5.74 (s), 5.76 (s)	5.73 (s), 5.75 (s)
CO	162.1	162.1		
Dehydroalanine (4)				
NH			9.08 (s)	9.08 (s)
αC	134.6	134.6		
βCH_2	104.3	104.2	5.66 (s), 6.14 (s)	5.66 (s), 6.13 (s)
CO	165.0	165.0		
NH_2			7.51 (s), 7.94(s)	7.51 (s), 7.93 (s)

[a] ^{13}C NMR (100 MHz) at 60°C and ^1H NMR (400 MHz) at 30°C were measured in DMSO-d_6.
[b] Multiplicity and coupling constants in Hz are indicated in parentheses.

Fig. 1. Partial structures of sulfomycin II.

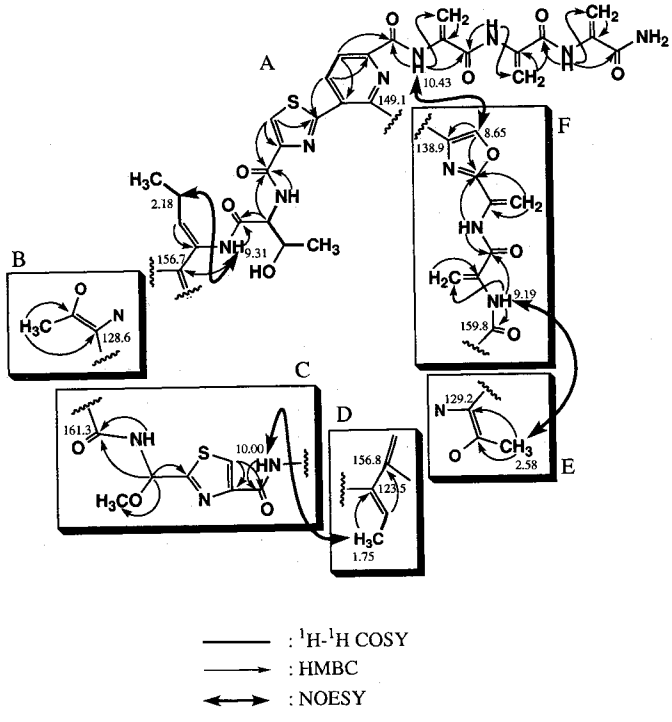

Fig. 2. Structures of sulfomycins I, II and III.

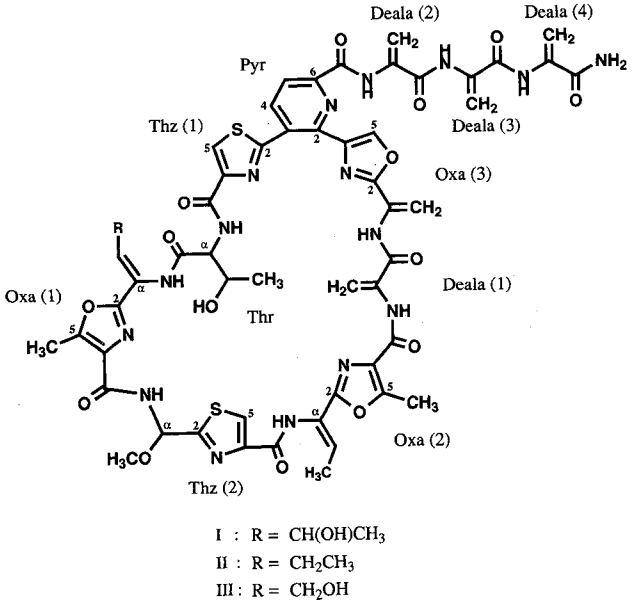

Table 2. Antimicrobial spectra of sulfomycin I, II and III and vancomycin.

Test organisms	MIC (μg/ml)			
	Sulfomycin			Vancomycin
	I	II	III	
Staphylococcus aureus 209P JC-1	0.1	0.2	0.78	0.78
Staphylococcus aureus Smith	0.2	0.39	1.56	1.56
MRSA TK731P	0.05	0.1	0.39	0.78
MRSA 252R	0.1	0.2	1.56	0.78
MRSA H-2-3	0.1	0.39	1.56	1.56
Staphylococcus epidermidis Kawamura	0.2	0.39	1.56	1.56
Enterococcus faecalis ATCC29212	0.1	0.2	1.56	3.13
Enterococcus faecium 173-6	0.05	0.1	1.56	1.56
Escherichia coli NIHJ JC-2	>100	>100	>100	>100
Klebsiella pneumoniae PCI-602	>100	>100	>100	>100
Pseudomonas aeruginosa 35R	>100	>100	>100	>100

MIC values were determined by agar dilution method.

CH_2–CH=), ^{1}H-^{13}C long range correlations observed in the HMBC spectrum exhibited the presence of six partial structures, **A** to **F**, as shown in Fig. 1. The connectivities of these partial structures were established by the observation of NOEs between the olefinic proton (δ 8.65) and the amide proton (δ 10.43), the amide proton (δ 9.19) and the methyl proton (δ 2.58), and the methyl proton (δ 1.75) and the amide proton (δ 10.00).

The ^{1}H and ^{13}C NMR data along with the deduced partial structures of sulfomycin II, clearly indicated the presence of an oxazole ring in **B** and **E**, respectively, in comparison with the corresponding NMR data of sulfomycin I[2]. The configurations of the >C=CH–CH_2CH_3 unit of **A** and >C=CH–CH_3 unit of **D** were both revealed to be *Z* by the observations of NOEs between the γ-methylene proton (δ 2.18) and the amide proton (δ 9.31), and between the γ-methyl proton (δ 1.75) and the amide proton (δ 10.00), respectively. The absolute configuration of the threonine unit was established to be L by analysis of the acidic hydrolysate of sulfomycin II on chiral-TLC. From these results, the structure of sulfomycin II was established as shown in Fig. 2.

The ^{1}H and ^{13}C NMR data for sulfomycin III are consistent with those of sulfomycin II except for γ-methylene and δ-methyl signals of Oxa (1). In sulfomycin III, the δ-methyl signal (δ_C 12.7 and δ_H 1.01) in sulfomycin

II was lacking, and an additional hydroxyl proton at δ4.92 was observed along with the downfield shift of γ-methylene signal in Oxa (1). Thus, the structure of sulfomycin III was determined as shown in Fig. 2.

The antibacterial activities of sulfomycins I, II and III and vancomycin are shown in Table 2. Sulfomycins II and III as well as sulfomycin I strongly inhibited the growth of Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* in comparison with those of vancomycin, but are not active against Gram-negative bacteria.

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