NOTE

Two novel nucleosidyl–peptide antibiotics: Sansanmycin F and G produced by *Streptomyces* sp SS

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Sansanmycins were a group of nucleosidyl–peptide antibiotics, produced by *Streptomyces* sp SS (CGMCC no. 1764).^{1–4} During our further studies on minor components of Sansanmycins, two new components, Sansanmycin F and G, were obtained. In this paper, we report the production, isolation, structure elucidation and biological activities of Sansanmycin F and G (Figure 1).

Streptomyces sp SS was cultured as described previously.¹ Antibiotic activity was determined by a paper-disk agar diffusion assay using *Pseudomonas aeruginosa* on Mueller–Hinton medium. The fermentation broth was filtered to remove mycelia; 50 l of filtrate thus obtained was applied on a column of Amberlite XAD-2 (Rohm and Haas Co., Philadelphia, PA, USA) (5 l) and, after washing with 25 l of water and 20 l of 10% aqueous acetone successively, the absorbed materials were eluted with 20 l of 30% aqueous acetone. The active fraction was concentrated *in vacuo* and lyophilized to obtain a crude powder (30.0 g). Thereafter, 5 g of the crude powder was dissolved in 100 ml 0.1% (w/v) (NH₄)₂CO₃ aqueous solution, and then applied on a column of Amberlite XAD1180 (1.25 l). After washing with 15 l of 8% aqueous acetone, the column was sequentially eluted with 15% (10 l) and 30% (10 l) of aqueous acetone to yield 100 fractions, each of which was analyzed by HPLC–UV (XTerra MS C18 column (Waters, Dublin, Ireland), 150×4.6 mm, 5 µm; MeOH/0.1% (w/v) (NH₄)₂CO₃, 3:7 (v/v); flow rate, 1 ml min^{−1}; UV detection at 254 nm and oven temperature at 40 °C). Fractions 60–80, containing Sansanmycin F and G, were combined, concentrated *in vacuo* and lyophilized to obtain a mixture of Sansanmycin F and G (100 mg). Further separation was achieved by means of preparative RP–HPLC (Shim-pack PREP-ODS column (Shim-pack, Kyoto, Japan), 250×20 mm, 10 µm; eluted with a gradient of buffer A (0.1% (w/v) (NH₄)₂CO₃–MeOH, 65:35 (v/v)) to buffer B (0.1% (w/v) (NH₄)₂CO₃–MeOH, 55:45 (v/v); flow rate, 5 ml min^{−1}; UV detection at 254 nm and oven temperature at 40 °C) to yield Sansanmycin F (15 mg) and G (10 mg).

Figure 1 Structures of Sansanmycin F and G.

Sansanmycin F is a white amorphous powder; electrospray ionization (ESI)–MS m/z 904.6 [M+H]⁺, HR-ESI-MS m/z 904.36496 [M+H]⁺ (calcd for C₄₃H₅₄N₉O₁₁S, 904.36635). ¹H and ¹³C NMR spectra of Sansanmycin F (Table 1) were similar to those of Sansanmycin,¹ except that there were new signals in the NMR spectra of Sansanmycin F instead of *m*-tyrosine residue in Sansanmycin. A series of NMR (¹H NMR, ¹³C NMR, ¹H–¹H COSY, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC)) data indicated that it had the 1,1-dimethyl-6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (TIC) (Figure 2) as the blocked N terminus, similar to Napsamycin B,⁵ which had 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid, and Mureidomycin F,⁶ which had 6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid as the blocked N terminus. The proton resonances at δ 6.75 and 7.18 were

Table 1 ^1H and ^{13}C NMR Data for Sansanmycin F and G in D_2O^a (pD=8.0, δ in p.p.m.)

Position ^b	Mult.	Sansanmycin F				Sansanmycin G			
		δ_{C}	δ_{H}	Extra signals due to conformers		δ_{C}	δ_{H}	Extra signals due to conformers	
				δ_{C}	δ_{H}			δ_{C}	δ_{H}
Uracil-2	N-CO-N	155.0				161.1		161.0	
Uracil-4	CO-N	170.0		170.5		178.4			
Uracil-5	CH	105.1	5.43		5.82	105.7	5.36	105.6	5.79
Uracil-6	CH	142.5	7.13		7.35	141.4	6.98		7.24
Sugar-1	O-CH-N	96.3	6.07	96.4	6.00	96.0	6.13		
Sugar-2	O-CH	75.5	4.44	75.6	4.47	75.3	4.50	75.4	
Sugar-3	CH2	35.8	2.91			35.9	2.68		
			2.94				3.00		
Sugar-4	>C=	147.1				147.3			
Sugar-5	-CH=	99.9	6.02	99.1	5.99	99.4	6.00		
AMBA-1	CO-N	170.5				170.3		170.1	
AMBA-2	CH	58.3	4.60			58.5	4.58		
AMBA-3	CH	53.3	4.96	59.2	4.35	53.2	4.96		4.81
AMBA-4	CH3	16.0	1.18	17.0	1.21	15.9	1.18		1.12
AMBA-N-CH3	N-CH3	32.6	2.95	31.2	2.82	32.6	2.94		
Trp-1	-COOH	182.1				182.2			
Trp-2	CH	59.2	4.38			59.1	4.37		
Trp-3	CH2	31.2	3.25			31.2	3.26		
			3.08				3.04		
Trp-2'	CH	126.9	7.18			127.0	7.19		
Trp-3'	ArC	113.3				113.3			
Trp-3a'	ArC	130.2				130.2			
Trp-4'	ArCH	121.5	7.62			121.6	7.66		
Trp-5'	ArCH	121.9	7.10			121.9	7.14		
Trp-6'	ArCH	124.4	7.17			124.5	7.41		
Trp-7'	ArCH	114.4	7.39			114.5	7.43		
Trp-7a'	ArC	138.9				138.9			
Ureido	N-CO-N	161.3				161.4			
TIC-carbonyl	CO-N	176.8				177.6			
TIC-1	C	56.5				56.1			
TIC-3	CH	51.9	4.01		4.35	51.8	3.92		
TIC-4	CH2	34.7	2.75			35.1	2.68		
			2.67				2.53		
TIC-5	ArCH	117.6	6.49		6.62	118.8	6.42		6.52
TIC-6	Ar-C-O	156.7				160.8			
TIC-7	ArCH	117.3	6.75			118.7	6.68		
TIC-8	ArCH	130.1	7.18	130.2		130.2	7.14		
TIC-9	ArC	137.1				135.4			
TIC-10	ArC	136.1				136.4			
TIC-1-methyl	CH3	32.7	1.46		1.50	33.2	1.43		
TIC-1-methyl	CH3	32.1	1.35		1.41	32.2	1.33		
Met-1/Leu-1 ^c	CO-N	177.5				178.7			
Met-2/Leu-2 ^c	CH	55.9	4.26			55.6	4.15		4.08
Met-3/Leu-3 ^c	CH2	33.8	1.90			43.2	1.43		
			1.82						
Met-4/Leu-4 ^c	CH	32.2	2.44			27.1	1.46		
			2.42						
Leu-5	CH ₃					23.9	0.83		
Met-6/Leu-6 ^c	CH ₃	17.0	2.02		1.95	23.8	0.82		

^aNMR data were recorded at 600 MHz for proton and at 150 MHz for carbon.^bAbbreviations for the structure units are as follows: AMBA, 2-amino-3-methylaminobutyric acid; Leu, leucine; Met, methionine; TIC, 1,1-dimethyl-6-hydroxy-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid; Trp, tryptophan.^cMet was for Sansanmycin F and Leu was for Sansanmycin G.

coupled (^1H - ^1H COSY) and corresponded to positions of 7 and 8 of TIC, respectively. The signals at δ 117.6, 137.1 and 156.7 were all coupled (HMBC) to the proton of TIC-7 and were assigned as carbons

of 5, 9 and 6 of TIC, respectively. The carbonyl that resonated at δ 176.8 and coupled to the proton of the *N*-methyl protons was assigned as TIC carbonyl. Also coupled to TIC carbonyl were the

protons at δ 4.01 (TIC-3) and δ 2.67 (TIC-4). The signal at δ 136.1, which was coupled to the protons of TIC-4 and TIC-3, was assigned as the carbon of TIC-10. The signal at δ 56.5 showing a cross-peak with protons of TIC-3 and TIC-8 was assigned as the carbon of TIC-1. The methyl proton resonances at both δ 1.46 and 1.35 were coupled to the carbons of TIC-1 and TIC-9, and corresponded to the two methyls of TIC-1. From these results, the structure of Sansanmycin F was elucidated as shown in Figure 1.

Figure 2 Key correlations of TIC moiety in HMBC and ^1H - ^1H COSY spectra of Sansanmycin F.

The total structure of Sansanmycin F was further confirmed by analysis of ESI-MS/MS spectra. The ESI-MS/MS data of the protonated molecular ion, m/z 904 (Figure 3), indicated that sequence selective fragmentations observed for Sansanmycin F showed complete similarity to those of sansanmycin,¹ except those originating from the N terminus. The fragment ion m/z 176 also confirmed that Sansanmycin F had TIC as the N terminus.

Sansanmycin G is a white amorphous powder; ESI-MS m/z 886.7 $[\text{M}+\text{H}]^+$, HR-ESI-MS m/z 886.40338 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{44}\text{H}_{56}\text{N}_9\text{O}_{11}$, 886.40993). Close examination of the ^1H and ^{13}C NMR data of Sansanmycin G suggested that it is similar to Sansanmycin F with a leucine moiety in place of the methionine unit. HPLC analyses of the alkaline hydrolysate (6N NaOH, 110°C , 16 h) of Sansanmycin G confirmed the presence of leucine residue. A series of 2D NMR experiments (^1H - ^1H COSY, HMQC and HMBC) were performed, which allowed assignment of all ^1H and ^{13}C signals associated with each amino-acid unit and pseudo nucleoside residue (Table 1). The total structure of Sansanmycin G (Figure 1) was also further confirmed by analysis of ESI-MS/MS spectra (Figure 3).

Sansanmycin F and G had weak antibacterial activity, as shown in Table 2. Sansanmycin F exhibited inhibitory activity against *P. aeruginosa* with an MIC value of $16\text{ }\mu\text{g ml}^{-1}$. Sansanmycin F and

Table 2 The MIC values of Sansanmycins

Test organism	MIC ($\mu\text{g ml}^{-1}$)				
	A*	F*	G*	INH	RFP
<i>M. tuberculosis</i> H ₃₇ Rv ATCC 27294	16	>16	>16	0.05	0.05
<i>M. phlei</i> 519	128	256	128	ND	ND
<i>P. aeruginosa</i> 11	8	16	>256	ND	ND
<i>Staphylococcus aureus</i> ATCC 90124	128	256	256	ND	ND
<i>Escherichia coli</i> ATCC 11775	256	>256	>256	ND	ND

Abbreviations: A, Sansanmycin; F, Sansanmycin F; G, Sansanmycin G; ND, not detected.

Figure 3 ESI-MS/MS data of Sansanmycin F and G.

G were further evaluated for their inhibitory activity against *Mycobacterium tuberculosis* H₃₇Rv, on the basis of alarm blue microplate assay,⁷ with MIC values more than 16 µg ml⁻¹.

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