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NOTE

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A New Nucleosidyl-peptide Antibiotic, Sansanmycin

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Abstract A new nucleosidyl-peptide antibiotic, sansanmycin, was isolated from an unidentified *Streptomyces* sp SS. The structure of sansanmycin was elucidated by analyses of its alkaline hydrolysate and spectroscopic analyses. Sansanmycin exhibits antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Ra$ and *Pseudomonas aeruginosa* with MIC values of 10 and 12.5 μg/ml, respectively.

Keywords Sansanmycin, nucleosidyl-peptide, antibacterial activity, *Mycobacterium tuberculosis* $H_{37}Ra$, *Pseudomonas aeruginosa*

In the course of a screening program for new antiobiotics, we found a new nucleosidyl-peptide antibiotic, sansanmycin, from an unidentified *Streptomyces* sp SS (CGMCC No. 1764). Sansanmycin inhibited not only *Pseudomonas aeruginosa* but also *Mycobacterium tuberculosis* $H_{37}Ra$. Chemical and spectroscopic analyses revealed that it belonged to the class of nucleosidyl-peptide antibiotics represented by the pacidamycins [1～3], mureidomycins [4～6] and napsamycins [7]. Herein we describe the fermentation, isolation, structural elucidation, and antibacterial activity of sansanmycin.

The unidentified *Streptomyces* sp SS was isolated from a soil sample obtained from Guizhou, China. Sporulating slant cultures of the strain SS grown on ISP 2 medium were used to inoculate into 500-ml Erlenmeyer flasks which contained 100 ml of a production medium consisting of glucose 1.5%, corn steep liquor 0.5%, peptone 0.3%, NaCl 0.4%, $(NH_4)_2SO_4$ 0.5%, $CaCO_3$ 0.3% (pH 7.5). The inoculated flasks were incubated on a rotary shaker (220 rpm) at 27°C for 96 hours. Antibiotic activity was determined by a paper-disk agar diffusion assay using *P. aeruginosa* on Mueller-Hionton medium. The fermentation broth was filtered to remove the mycelia, 20 liters of filtrate thus obtained was applied on a column of Amberlite XAD-2 (2 liters), and after washing with 10 liters of water and 8 liters of 20% aqueous methanol successively, the adsorbed materials were was eluted with 8 liters of 50% aqueous methanol. The active fraction was concentrated *in vacuo* and lyophilized to obtain a crude powder (15.2 g). Then 5 g of the crude powder was dissolved in 10 ml of buffer A (0.1% (w/v) $(NH_4)_2CO_3$ - MeOH, 1 : 4 (v/v)), and then was applied to an ODS (YMC Gel, ODS-A, 12 nm, S-150 μm) column (50×3 cm, 353 ml) which was pre-equilibrated in buffer A. The column was sequentially eluted at 3 ml/minute with buffer A (0.3 liters), followed by a gradient of buffer A to buffer B (0.1% (w/v) $(NH_4)_2CO_3$ - MeOH, 1 : 1 (v/v)) over 1.5 liters. The bioactive fractions

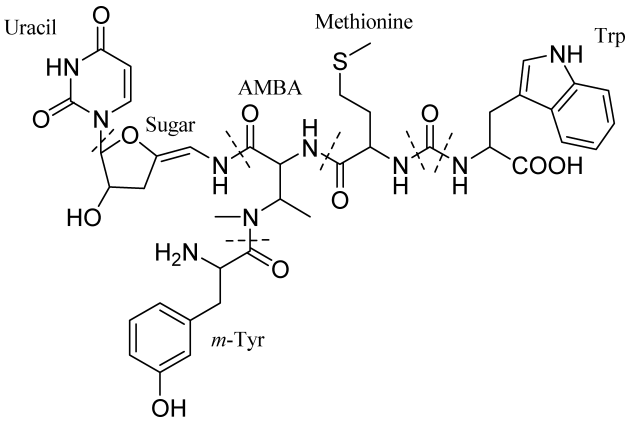

Fig. 1 Structure of sansanmycin.

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Table 1 ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for sansanmycin

Position*	^{13}C shift	^{1}H shift	Multiplicity	Extra signals due to conformers	
				^{13}C shift	^{1}H shift
Uracil-2	154.4		N–CO–N	154.6	
Uracil-4	169.6		CO–N	170.0	
Uracil-5	105.1	5.40	CH		5.82
Uracil-6	143.2	7.28	CH	142.3	6.86
Sugar-1	96.0	5.90	O–CH–N	96.3	5.97
Sugar-2	75.6	4.38	O–CH	75.3	4.15
Sugar-3	35.6	2.82	CH_2	35.8	2.70
					2.56
Sugar-4	147.5		>C=	146.9	
Sugar-5	99.8	5.92	–CH=	99.4	5.97
AMBA-1	170.6		CO–N	170.2	
AMBA-2	58.5	4.61	CH	58.9	4.49
AMBA-3	53.6	4.91	CH	56.2	4.13
AMBA-4	16.1	1.17	CH_3	16.6	0.57
AMBA-N–CH_3	32.8	2.93	N–CH_3	30.8	2.65
m-Tyr-1	177.8		CO–N	179.0	
m-Tyr-2	54.8	4.04	CH	54.4	4.28
m-Tyr-3	41.9	2.50	CH_2	43.3	2.67
		2.87			2.87
m-Tyr-1′	140.9		ArC		
m-Tyr-2′	118.9	6.71	ArCH		6.68
m-Tyr-3′	158.9		Ar-C–O		
m-Tyr-4′	117.2	6.77	ArCH		
m-Tyr-5′	133.2	7.21	ArCH		
m-Tyr-6′	124.0	6.72	ArCH		
Trp-1	182.2		–COOH		
Trp-2	59.1	4.38	CH		
Trp-3	31.2	3.24	CH_2		
		3.07			
Trp-2′	126.9	7.14	CH		
Trp-3′	113.2		ArC	113.3	
Trp-3a′	130.2		ArC		
Trp-4′	121.6	7.59	ArCH		
Trp-5′	121.9	7.07	ArCH		
Trp-6′	124.5	7.14	ArCH		
Trp-7′	114.5	7.40	ArCH		
Trp-7a′	138.9		ArC		
Ureido	161.1		N–CO–N	161.2	
Methionine-1	177.4		CO–N	177.5	
Methionine-2	55.7	4.26	CH		
Methionine-3	33.8	1.87	CH_2		
		1.79			
Methionine-4	32.1	2.41	CH_2		
Methionine-*S*-CH_3	17.0	1.98	CH_3		

The spectra were recorded in slightly alkaline D_2O (pD 8). The chemical shifts (δ) are given in ppm.
* Abbreviations for the structure units are: *m*-Tyr=*m*-tyrosine, Trp=tryptophan, AMBA=2-amino-3-methylaminobutyric acid.

were analyzed by HPLC (SHIMADZU VP-ODS column, 150×4.6 mm; 40% MeOH; flow rate, 0.7 ml/minute; UV detection at 254 nm; oven temperature, 40°C). The fractions containing sansanmycin were combined, desalted by adsorption on an X-5 macropore resin (Nankai Chemicals, China) column, and concentrated *in vacuo* to give a white powder of sansanmycin (200 mg).

Sansanmycin 1: whiter powder; UV λ_{max}^{MeOH} nm 221, 258, 290; FAB-MS *m*/*z* 886 $[M+Na]^+$, 908 $[M+Na+Na-H]^+$, ESI-MS *m*/*z* 864.4 $[M+H]^+$, HR-ESI-MS *m*/*z* 864.33342 $[M+H]^+$ (calcd for $C_{40}H_{50}N_9O_{11}S$, 864.33505). See Table 1 for NMR spectral data. The NMR data were recorded in slightly alkaline D_2O (pD 8) to partly simplify the spectra which were complicated by peak doubling due to rotational conformers about the amide bonds. This phenomenon is often observed in peptides especially those containing *N*-methylated amino acids or proline. The ESI-MS/MS data of the parent ion peak (*m*/*z* 864) are shown in Fig. 2. The structure of sansanmycin was deduced from these data as well as analyses of the alkaline hydrolysate.

GC-MS analyses of the alkaline hydrolysate (4 N NaOH, 110°C, 18 hours) of sansanmycin as their trifluoroacetyl *n*-butyl ester derivatives indicated the presence of methionine, *m*-tyrosine, tryptophan and 2-amino-3-methylaminobutyric acid (AMBA). The presence of an unusual nucleoside moiety in the molecular was suggested by the ^1H and ^{13}C NMR data for sansanmycin, including results from COSY, DEPT, HSQC and HMBC experiments, in which the chemical shift data were in accordance with reported values of those of pacidamycin D [3] except for the peak doubling.

The total structure of sansanmycin was established by the analysis of the ESI-MS/MS spectrum of the protonated molecular ion, *m*/*z* 864 (Fig. 2). Sequence selective fragmentations observed for sansanmycin showed complete similarity to those reported in the FAB-MS/MS spectrum of mureidomycin A except those originating from the *C*-terminus. The fragment ions at *m*/*z* 205 and 660 observed for sansanmycin indicated that it possessed a tryptophan residue at the *C*-terminus. The presence of the uracil unit in sansanmycin was revealed by the fragment ion corresponding to loss of *m*/*z* 112 from the daughter ion (*m*/*z* 846, loss of H_2O from the parent ion). The total structure of sansanmycin was thus elucidated as shown in

Table 2 The MIC of sansanmycin

Test organism	MIC (μg/ml)
Mycobacterium tuberculosis $H_{37}Ra$	10
Pseudomonas aeruginosa ATCC 10145	12.5
Staphylococcus aureus ATCC 90124	200
Escherichia coli ATCC 11775	200

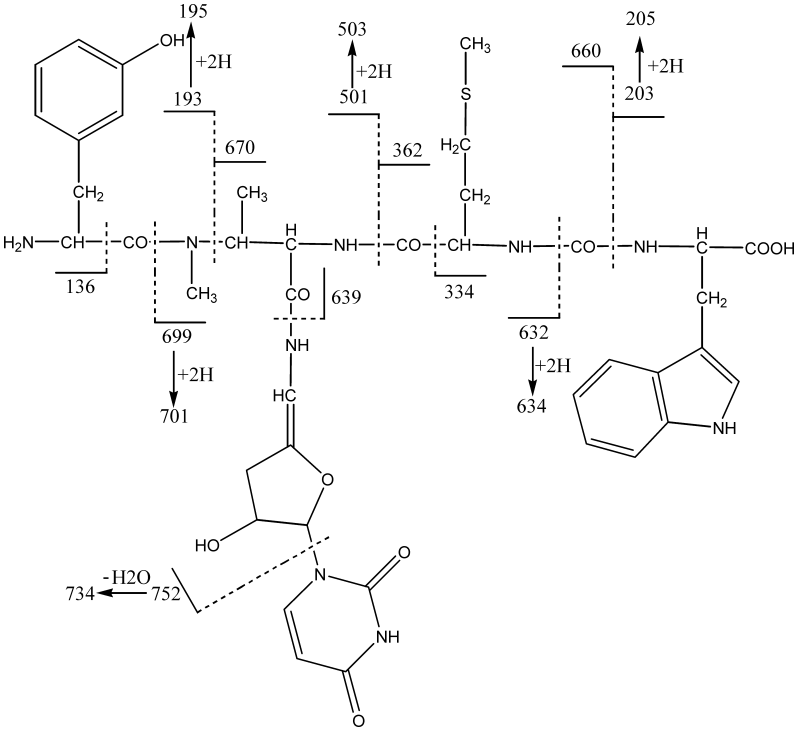

Fig. 2 The ESI-MS/MS data of parent ion peak (*m*/*z* 864) of sansanmycin.

Fig. 1.

Sansanmycin is active against *M. tuberculosis* $H_{37}Ra$ and *P. aeruginosa* with MIC values of 10 and 12.5 μg/ml, respectively, but has very poor activity against other Gram-negative and Gram-positive bacteria, MIC values being higher than 200 μg/ml (Table 2).

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