TMC-135A and B, New Triene-ansamycins, Produced by *Streptomyces* sp.

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In the course of screening for new bioactive compounds, we have isolated two antibiotics from the fermentation broth of *Streptomyces* sp. TC 1190 and were designated TMC-135A and B. Spectroscopic analyses revealed that TMC-135s were new members of triene-ansamycins, closely related to thiazinotrienomycins and cytotrienins[1~3]. This paper briefly describes the taxonomy, isolation, physico-chemical properties, structure elucidation and biological activity of TMC-135A and B.

The producing strain TC 1190 was isolated from a soil sample. The substrate mycelium developed well and branched irregularly. Each spore chain, which was spiral type, had 20 to 50 or more spores per chain. The spores were cylindrical, with size of 0.5~0.8×0.6~0.9 μm, and the surface was rugose. Fragmentation of substrate mycelium, sporangium, or motile spore was not observed. Analysis of the whole-cell hydrolysates of the strain showed the presence of LL-diaminopimelic acid. On the basis of these morphological and chemotaxonomic characteristics, strain TC 1190 was assigned to the genus *Streptomyces*.

Streptomyces sp. TC 1190 was inoculated into a 500-ml Erlenmeyer flask containing 70 ml of a seed medium composed of 6% dextrin, 3% soybean flour, 0.2% yeast extract, 0.05% L-tryptophan, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.02% $(NH_4)_2HPO_4$. The medium was adjusted to pH 7.0 before autoclaving. The culture was incubated for 6 days at 27°C on a rotary shaker (220 rpm). One milliliter of the seed culture was transferred to a 500-ml Erlenmeyer flask containing 70 ml of a production medium composed of 6% dextrin, 3% soybean meal, 0.2% yeast extract, 0.05% L-tryptophan, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.02% $(NH_4)_2HPO_4$. The medium was adjusted at pH 7.0 before autoclaving. The culture was incubated for 6 days at 27°C on a rotary shaker (220 rpm).

The fermentation broth (30 liters) of strain TC 1190 was extracted with 1-butanol (15 liters). The extract was concentrated and then dissolved in water (0.8 liter) and extracted twice by ethyl acetate (0.8 liter each). The ethyl acetate layer was concentrated to dryness under reduced pressure, and applied on a silica gel column chromatography developed with CH_2Cl_2 - CH_3OH (95 : 5). The fraction containing TMC-135A was collected and concentrated (2 g), and then subjected to Sephadex LH-20

Table 1. Physico-chemical properties of TMC-135A and B.

	TMC-135A	TMC-135B
Appearance	Pale yellow powder	Pale yellow powder
MP (dec.)	166 ~ 168 °C	190 ~ 192 °C
ESI-MS (*m/z*)	742 $(M+Na)^+$, 758 $(M+K)^+$	742 $(M+Na)^+$, 758 $(M+K)^+$
HRESI-MS (*m/z*)		
Found	718.3143 $(M-H)^-$	718.3143 $(M-H)^-$
Calcd.	718.3162 for $C_{39}H_{48}N_3O_8S$	
Molecular formula	$C_{39}H_{49}N_3O_8S$	$C_{39}H_{49}N_3O_8S$
UV λ_{max} (MeOH) nm (log ε)	216 (4.42), 259 (4.50), 270 (4.46), 282 (4.34), 310 (3.33)	217 (4.46), 259 (4.63), 270 (4.58), 281 (4.43), 310 (3.56)
IR ν_{max} (KBr) cm^{-1}	3400, 2930, 1720, 1665, 1520 1470, 1370, 1180, 1000	3400, 2930, 1720, 1665, 1520 1470, 1375, 1180, 1000
HPLC Rt (minute)[a]	41	39

[a] See text.

column with CH_2Cl_2-CH_3OH (1 : 1). The active eluate was concentrated and purified on a reverse phase silica gel (YMC ODS A60) column developed with 50% aqueous acetonitrile. Final purification of TMC-135A was achieved by preparative HPLC (column, YMC-D-ODS-5 30×250 mm; mobile phase, 42% aqueous acetonitrile; flow rate, 25 ml/minute; detection, UV 254 nm; Rt, 41 minutes) to give TMC-135A (14 mg). The fraction of the silica gel column chromatography containing TMC-135B (3.1 g), was similarly processed by Sephadex LH-20, YMC ODS, and the HPLC to afford pure TMC-135B (16 mg). The physico-chemical properties of TMC-135A and B are listed in Table 1.

TMC-135A and B showed similar physico-chemical properties to each other. Both of the molecular formulas of TMC-135A and B were established as $C_{39}H_{49}N_3O_8S$ on the basis of high-resolution ESI-MS, and ^1H and ^{13}C NMR spectral data. The UV absorption at 259, 270, and 281 nm

Table 2. ^{13}C and ^1H NMR data for TMC-135A and B.

	TMC-135A[a]		TMC-135B[b]	
Position	δ_C	δ_H	δ_C	δ_H
1	171.2 s		170.6 s	
2	42.5 t	2.72, 2.98 (2H, dd)	43.5 t	2.93, 3.17 (2H, m)
3	79.2 d	4.25 (1H, m)	80.8 d	4.47 (1H, m)
4	129.5 d	5.54 (1H, dd, 7.3, 15.4)	131.6 d	5.73 (1H, dd, 8.7, 15.3)
5	135.1 d	6.29 (1H, dd, 10.5, 15.4)	135.4 d	6.58 (1H, dd, 9.5, 15.3)
6	129.6 d	6.08 (1H, dd, 10.5, 14.9)	129.9 d	6.34 (1H, dd, 9.5, 14.7)
7	134.9 d	6.23 (1H, dd, 10.6, 14.9)	134.8 d	6.34 (1H, dd, 9.4, 14.7)
8	133.9 d	6.06 (1H, dd, 10.6, 15.1)	133.7 d	6.20 (1H, dd, 9.4, 15.3)
9	130.4 d	5.65 (1H, m)	130.8 d	5.89 (1H, m)
10	34.3 t	2.35, 2.65 (2H)	33.4 t	2.32, 2.93 (2H, m)
11	76.3 d	4.81 (1H, m)	76.2 d	5.22 (1H, m)
12	39.3 d	1.78 (1H, m)	38.9 d	2.19 (1H, m)
13	68.4 d	4.51 (1H, br-s)	68.0 d	5.08 (1H, br-s)
14	138.8 s		140.6 s	
15	123.3 d	5.05 (1H, m)	123.8 d	5.41 (1H, br-d)
16	24.6 t	2.29 (2H, m)	27.1 t	2.62 (2H, m)
17	32.1 t	2.08, 3.10 (2H, m)	29.8 t	3.04, 3.17 (2H, m)
18	132.3 s		129.8 s	
19	146.5 s		144.2 s	
20	123.0 s		126.7 s	
21	112.8 s		109.6 d	7.54 (1H, s)
22	129.6 s		131.4 s	
23	118.4 d	6.68 (1H, s)	118.3 s	
24	30.2 t	3.36, 3.46 (2H, 14.8)	30.7 t	3.53, 3.60 (2H, d, 14.7)
25	165.6 s		165.9 s	
26	9.9 q	0.78 (3H, d, 6.8)	10.1 q	0.87 (3H, d, 6.8)
27	20.9 q	1.74 (3H, s)	21.4 q	2.03 (3H, s)
28	57.1 q	3.42 (3H, s)	56.2 q	3.28 (3H, s)
29	173.2 s		172.9 s	
30	34.4 s		34.5 s	
31	18.4 t	1.40, 1.72 (2H, m)	17.4 t	1.37, 1.81 (2H, m)
32	18.1 t	1.21, 1.57 (2H, m)	17.4 t	1.24, 1.60 (2H, m)
33	170.0 s		171.4 s	
34	133.1 s		134.3 s	
35	135.5 d	6.74 (1H, br-s)	134.0 d	6.89 (1H, s)
36	26.0 t	2.20 (2H, m)	25.7 t	2.13 (2H, br-s)
37	21.9 t	1.67 (2H, m)	22.1 t	1.63 (2H, m)
38	22.5 t	1.74 (2H, m)	22.6 t	1.65 (2H, m)
39	26.3 t	2.13 (2H, m)	24.7 t	2.60 (2H, m)
13-OH		2.85 (br-s)		5.23 (br-s)
30-NH		6.41 (s)		9.52 (s)
19-OH		7.68 (s)		9.63 (br-s)
20-NH		8.01 (s)		11.00 (s)
22-NH		8.38 (s)		11.79 (s)

[a] Recorded in $CDCl_3$ at 100 and 400 MHz, respectively

[b] Recorded in pyridine-d_5 at 100 and 400 MHz, respectively

together with the IR absorption at 1000 cm^{-1} indicated the presence of a triene structure in TMC-135A and B. The presence of an ester (1720 cm^{-1}) and an amide (1665 cm^{-1}) group was also suggested by the IR spectra. The physicochemical properties, described above, strongly indicated that TMC-135s were new members of triene-ansamycins.

The ^{13}C and ^{1}H NMR data of TMC-135A and B are summarized in Table 2. The ^{13}C and ^{1}H NMR spectra displayed 39 carbon and 49 proton signals, respectively. The structures of TMC-135A and B were elucidated by NMR studies, comparing with the data of thiazinotrienomycins and cytotrienins[1~3]. The major correlations observed in COSY, HMBC and selective INEPT (optimized at 3 Hz) are shown in Fig. 1. As a result, TMC-135A was deduced to be a hybrid of thiazinotrienomycin A and cytotrienin A, in which a D-alanine unit at C-11 of thiazinotrienomycin A was replaced with a 1-aminocyclopropane carboxylic acid. Similarly TMC-135B had the same cyclopropane side chain at C-11 of thiazinotrienomycin D, as illustrated in Fig. 1.

The cytotoxic activities of TMC-135A and B against several tumor cell lines, determined according to the methods previously described[4], are summarized in Table 3. TMC-135A and B showed strong cytotoxicities to various tumor cell lines.

In this study, we have isolated two new triene ansamycin antibiotics, TMC-135A and B. Interestingly, the cytotoxicitiy of TMC-135A was approximately 10 times stronger than that of TMC-135B, although the structure of TMC-135A was closely related to that of TMC-135B. The same phenomenon was observed in tiazinotrienomycins A and D[1]. The slight decomposition of TMC-135A in pyridine during the NMR measurement, might have some relation to the biological activity (no decomposition was observed for TMC-135B under the same condition). Recently, the mode of action of this class of antitumor ansamycins has been investigated. Cytotrienin A was reported to induce apoptosis of HL-60 cells *via* caspase-mediated activation of myelin basic protein kinase[5], and thiazinotrienomycin B against human stomach tumor SC-6

Fig. 1. Sturctures of TMC-135A and B elucidated by NMR analyses.

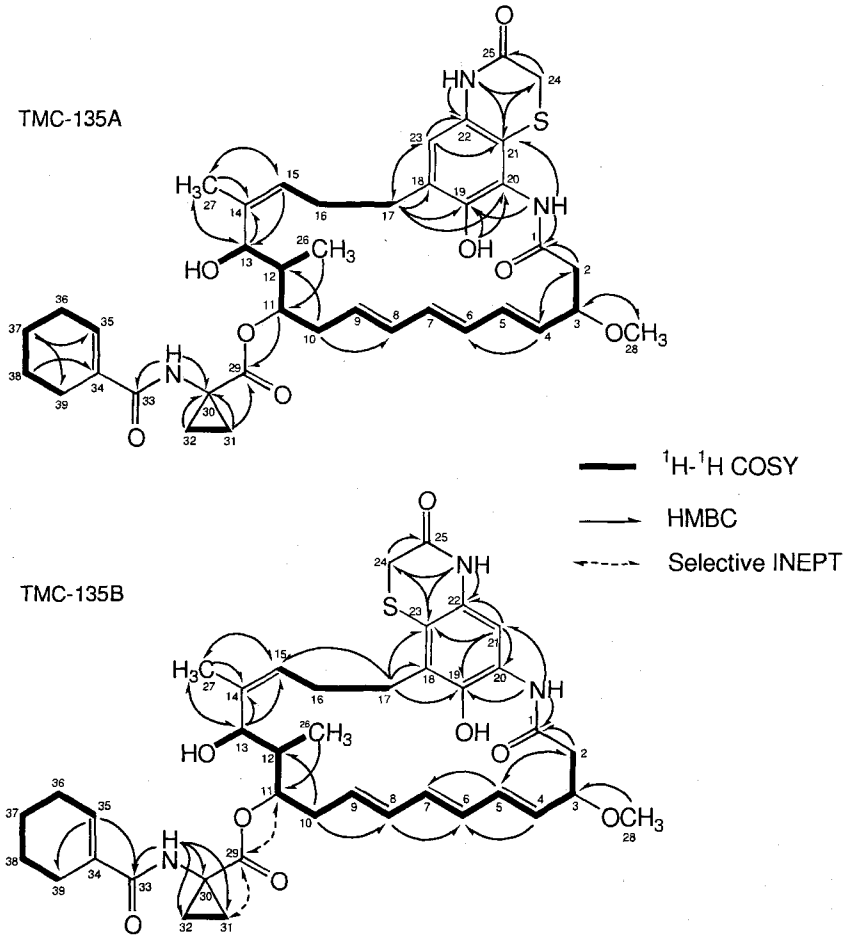

Table 3. Cytotoxicities of TMC-135A and B against tumor cells *in vitro*.

Cell lines	IC_{50} (μM)	
	TMC-135A	TMC-135B
HCT-116 human colon carcinoma	0.07	0.86
SK-BR-3 human breast adenocarcinoma	0.88	6.5
HeLa S3 human epitheloid carcinoma	0.11	1.1
U937 human histiocytic lymphoma	0.11	0.6
WiDr human colon adenocarcinoma	0.20	1.6
HT29 urinary bladder carcinoma	0.15	1.3
HL-60 human premyelocytic leukemia	0.05	0.30
THP1 human monocytic leukemia	0.06	0.32
Raji Burkitt's Lymphoma	0.05	0.34
Jurkat human lymphoma	0.13	0.47
P388D1 murine lymphoid neoplasm	0.56	2.5
B-16 murine melanoma	0.07	0.67

cells was revealed to interfere with the signal transduction of the epidermal growth factor receptor[6]. We hope TMC-135A and B will help further elucidation of the mechanism of this class of antibiotics.

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