STRUCTURAL STUDIES ON MINOR COMPONENTS OF TRIENOMYCIN GROUP ANTIBIOTICS TRIENOMYCINS D AND E

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In our study to discover antitumor antibiotics from microorganisms, ansamycin antibiotics, trienomycins A (**1**), B (**2**), and C (**3**) have been found in the culture broth of *Streptomyces* sp. No. 83-16 as strong cytotoxic compound against HeLa S_3 cells.[1] This study was undertaken to isolate further minor components from the broth. Trienomycins D (**4**) and E (**5**) were consequently isolated.

Fermentation

The inoculum for antibiotic production was prepared in a medium containing glucose 2%, peptone 0.5%, meat extract 0.5%, dry yeast 0.3%, NaCl 0.5%, $CaCO_3$ 0.3% (ajusted to pH 7.0 before sterilization). A 250-ml Erlenmeyer flask containing 30 ml of this medium was inoculated from a slant of *Streptomyces* sp. No. 83-16. The flask was incubated at 28°C on a rotary shaker at 220 rpm for 72 hours. Six ml of this seed culture was transferred to 300 ml of the same medium in 2-liter flasks and incubated as above for 48 hours. 1.5-liter of the second seed was used to inoculate a 30-liter jar fermentor containing 18 liters of the following medium: Glucose 4%, yeast extract 1.5%, soy bean meal 0.1%, NaCl 0.5%, $CaCO_3$ 0.3% in tap water (ajusted to pH 7.0 before sterilization). The fermentation was carried out for 72 hours at 28°C with an air flow of 15 liters per minute and an agitation rate of 250 rpm. Antibiotic production was monitored by HPLC.

Isolation

To the whole broth was added 8 liters of PrOH, and ajusted to pH 4.9 with conc HCl. The above mixture was filtered with an aid of Celite.

The filtrate (23 liters) was passed on Diaion HP-20 (2 liters) and the column was washed with 40% aq acetone and eluted with 65% aq acetone. After evaporation of solvent, the residue was extracted with EtOAc. The extract was concentrated to a small volume, and addition of *n*-hexane afforded crude trienomycin complexes. The crude complex was dissolved in acetone, applied to a column of Diaion HP-20SS (2 liters) and eluted with 50% aq acetone. The fractions of trienomycin A containing trienomycins D and E were concentrated, and extracted with EtOAc. The extract was concentrated to a small volume, and addition of *n*-hexane afforded trienomycin complexes as colorless powder. Further purification of trienomycins D (**4**) and E (**5**) was accomplished by preparative HPLC (YMC pack D-ODS-10 (20×250 mm)). The column was eluted with 65% aq MeOH as a mobile phase. The resulting fractions were monitored by HPLC (YMC pack A-312 ODS (6×150 mm)) using 65% aq MeOH as a mobile phase. The fractions containing pure trienomycin D (**4**) were combined, concentrated, and extracted with EtOAc. The extracts were concentrated to a small volume, to which *n*-hexane was added to give 5 mg of pure trienomycin D (**4**) as colorless powder. Fifteen mg of pure trienomycin E (**5**) was also isolated as the same method.

Structures of Trienomycins D and E

Trienomycin D (**4**) is a colorless powder which decomposes at 137~139°C. The UV spectrum is very similar to that of trienomycin A. The molecular formula of trienomycin D (**4**) was elucidated by high resolution mass spectrometry (HR-MS); observed: *m/z* 621.3574 (M^++H); calcd for $C_{36}H_{49}N_2O_7$: 621.3540. This molecular formula shows two hydrogen atom less than trienomycin A (**1**). The ^{13}C NMR spectrum of trienomycin D (**4**) was quite similar to that of trienomycin A (**1**) except the signal of attributed to acyl group attached to the alanyl moiety (Table 1). The ^{13}C NMR signals attributed to the acyl moiety of trienomycin D (**4**) were as follows; δ 168.7 (s), 132.2 (s), 135.5 (d), 24.1 (t), 21.5 (t), 22.1 (t), 25.5 (t). When the ^1H NMR spectra of trienomycins A (**1**) and D (**4**) were compared, a new signal appeared at 6.71 (1H, t, J=4.0 Hz). These observations in-

Table 1. ^{13}C NMR spectral data for trienomycins A, B, D, and E.

Carbon No.	A[1)]	D	B[1)]	E
C-1	170.8 (s)	168.7	168.9	168.8
C-2	44.8 (t)	43.6	43.6	43.5
C-3	81.6 (d)	78.7	79.1	78.7
C-4	132.5 (d)	130.6[a]	130.7	130.7[a]
C-5	135.2 (d)	134.2[b]	134.0	134.2[b]
C-6	131.0 (d)	129.5[a]	129.4	129.5[a]
C-7	135.0 (d)	133.6[b]	133.8	133.6[b]
C-8	134.6 (d)	133.4[b]	133.4	133.4[b]
C-9	130.5 (d)	129.3[a]	129.4	129.4[a]
C-10	33.7 (t)	33.2	33.3	33.3
C-11	76.4 (d)	75.5	75.6	75.5
C-12	40.4 (d)	39.7	39.6	39.7
C-13	69.7 (d)	68.4	68.5	68.5
C-14	139.7 (s)	138.4[c]	138.3	138.3[c]
C-15	125.9 (d)	124.8	125.0	125.1
C-16	30.8 (t)	29.6	29.3	29.5
C-17	37.3 (t)	36.3	36.3	36.3
C-18	144.9 (s)	144.1	144.0	144.1
C-19	112.9 (d)	111.0[d]	111.2	111.0[d]
C-20	140.2 (s)	138.6[c]	138.3	138.4[c]
C-21	107.2 (d)	105.9	106.1	106.0
C-22	158.6 (s)	157.3	157.3	157.4
C-23	113.4 (d)	112.0[d]	112.3	112.2[d]
C-24	10.2 (q)	9.8	9.9	9.9
C-25	20.8 (q)	20.3	20.3	20.3
C-26	56.6 (q)	56.8	56.7	56.7
C-27	173.3 (s)	173.0	172.9	172.9
C-28	50.5 (d)	48.8	48.7	48.7
C-29	17.2 (q)	17.9	17.6	17.8
C-30	179.2 (s)	168.7 (s)	173.3 (s)	174.0 (s)
C-31	45.9 (d)	132.2 (s)	45.4 (t)	34.4 (t)[e]
C-32	30.5 (t)	135.5 (d)	26.1 (d)	34.3 (t)[e]
C-33	26.7 (t)	24.1 (t)	22.4 (q)	27.8 (d)
C-34	26.9 (t)	21.5 (t)[e]	22.4 (q)	22.3 (q)
C-35	26.7 (t)	22.1 (t)[e]		22.3 (q)
C-36	30.5 (t)	25.5 (t)		

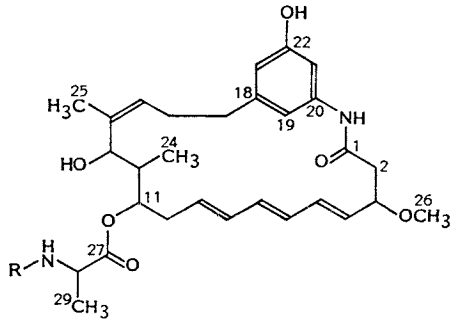
[a~e] Assignments may be exchangeable.

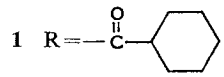
dicated that instead of the hexahydrobenzoyl moiety of trienomycin A (**1**), a tetrahydrobenzoyl moiety is attached to the alanyl moiety in trienomycin D (**4**). Trienomycin E (**5**) is a colorless powder which decomposes at 127~129°C. The UV and ^1H NMR spectra were very similar to that of trienomycin B (**2**). The molecular formula of trienomycin E (**5**) was elucidated by HR-MS; observed: *m/z* 611.3716 (M^++H); calcd for $C_{35}H_{51}N_2O_7$: 611.3697. This molecular formula shows CH_2 more than trienomycin B (**2**). The ^{13}C NMR spectrum of trienomycin E (**5**) was quite similar to that of trienomycin B (**2**) except the signal of attributed to acyl group attached to the alanyl moiety (Table 1). The ^{13}C NMR signals attributed to the acyl moiety of trienomycin E (**5**) were as follows; 174.0 (s), 34.4 (t), 34.3 (t), 27.8 (d), 22.3 (q, 2×C). These results indicated that the 4-methylpentyryl moiety is attached to the alanyl moiety of trienomycin E (**5**). From all of accumulated data described above, structure of trienomycins D (**4**) and E (**5**) were concluded to be **4** and **5** (Fig. 1), respectively.

Biological Properties of Trienomycins D and E

Trienomycins D (**4**) and E (**5**) were shown to

Fig. 1. Structures of trienomycins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**).





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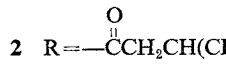

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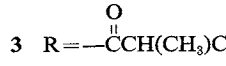

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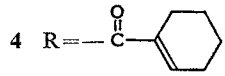

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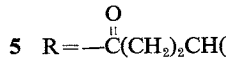

5 R=

exhibit cytotoxic activity against HeLa S_3 cells. When the cells were exposed to the antibiotics for 3 days, the growth of HeLa S_3 cells were inhibited at concentration of 0.022 μg/ml of trienomycin D (**4**) and 0.104 μg/ml of trienomycin E (**5**). Trienomycins D (**4**) and E (**5**) exhibited 2~5-fold weaker activity than trienomycin A (**1**).

Acknowledgment

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Reference

1) FUNAYAMA, S.; K. OKADA, K. IWASAKI, K. KOMIYAMA & I. UMEZAWA: Structures of trienomycins A, B and C, novel cytocidal ansamycin antibiotics. J. Antibiotics 38: 1677~1683, 1985, refs cited therein