NEW ANTIBIOTICS, ENAMINOMYCINS A, B AND C
II. PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES

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Physico-chemical characterization of enaminomycins revealed that these antibiotics are new members of the epoxy quinone family. From elementary analysis and mass spectroscopic measurements the molecular formulae of enaminomycins A, B and C appear to be $C_7H_5NO_5$, $C_{10}H_{11}NO_6$ and $C_7H_7NO_5$, respectively. They are very unique in their chemical properties, possessing various functions, such as epoxy, primary amine and carboxylic acid, in their small structural units.

Enaminomycin A, the most potent component, has activity against Gram-positive and Gram-negative bacteria and shows cytostatic effect on L1210 mouse leukemia cells *in vitro*, but enaminomycins B and C are only weakly active against Gram-positive and Gram-negative bacteria.

As described in the preceding paper,[1] enaminomycins are produced by *Streptomyces baarnensis* No. 13120. Fermentation and isolation studies indicated the presence of three antibiotics with very closely related structures. They were separated, purified and designated as enaminomycins A, B and C. In the present report, the physico-chemical and biological properties of these three antibiotics are described.

Physico-chemical Properties

Enaminomycin A was obtained as an amorphous powder after purification on a column of Sephadex LH-20 using ethyl acetate - methanol (95 : 5) as the solvent system. The reaction product of enaminomycin A with diazomethane, however, was crystallized from ethyl acetate - acetone to give colorless needles. Enaminomycins B and C were both obtained as colorless needles from ethyl acetate-acetone and methanol, respectively, after purification by the same column chromatographic procedure as used for A. Diazomethane treatment also yielded crystalline derivatives of enaminomycins B and C.

Enaminomycins A and B are soluble in water, methanol, ethanol and acetone, slightly soluble in chloroform and ethyl acetate and insoluble in *n*-hexane. The antibiotics reacted positively to 2,4-dinitrophenylhydrazine, ninhydrin, sulfuric acid and potassium permanganate. Enaminomycin C is soluble in water and methanol, slightly soluble in ethanol, acetone and ethyl acetate, and insoluble in *n*-hexane. The color reaction of enaminomycin C with the above reagents was the same as that of enaminomycins A and B. All three antibiotics behaved as acidic substances on high voltage paper electrophoresis (60 volts/cm, 3 mA/cm) at pH 7.5 for 30 minutes. The relative mobilities of A, B and C were 1.0, 0.72 and 0.84, respectively, when mobility of bromophenol blue was defined as 1.0. Their molecular weights and molecular formulae were derived from the analysis of high resolution mass spectrometry. These results as well as other physical and chemical properties are summarized in

Table 1. Physico-chemical properties of enaminomycins (EnM) A, B and C.

	EnM A	EnM B	EnM C
Appearance	Amorphous powder	Colorless needles	Colorless needles
m.p.	105°C (dec.)	160°C (dec.)	173°C (dec.)
$[\alpha]_D^{20}$	−20.9° (*c* 0.83, MeOH)	+60.1° (*c* 0.99, MeOH)	+31.4° (*c* 0.14, MeOH)
Elementary analysis (%)	Found C 47.20, H 3.84, N 7.33	C 49.72, H 4.60, N 5.58	C 44.97, H 3.72, N 7.17
	Calcd. C 45.91, H 3.75, N 7.65	C 49.79, H 4.56, N 5.81	C 45.51, H 3.81, N 7.57
Molecular formula	$C_7H_5NO_5$	$C_{10}H_{11}NO_6$	$C_7H_7NO_5$
Molecular ion (*m/e*)	183	241	185
λ_{max}^{MeOH} ($E_{1cm}^{1\%}$)	293 nm (530)	293 nm (580)	290 nm (930)
	245 nm (285)	244 nm (315)	245 nm (436)
ν cm^{-1} (KBr)	3315, 3200, 1730, 1710	3340, 3200, 3150, 1720, 1670, 1650	3400, 3240, 3180, 1680, 1635, 1605
δ ppm*	4.10 1H J=4.5 Hz	4.10 1H J=4.5 Hz	3.63 1H J=4.5 Hz
	4.15 1H J=4.5 Hz	3.60 1H J=4.5 Hz	3.82 1H J=4.5, 2 Hz
		3.15 2H S	4.81 1H J=2, 8.5 Hz
		2.20 3H S	6.56 1H J=8.5 Hz
Rf**	0.64	0.52	0.39
Color reaction	2,4-DNP, H_2SO_4+	2,4-DNP, H_2SO_4+	2,4-DNP, H_2SO_4+

* A and B in acetone-d_6, C in DMSO-d_6

** Eastman silica gel sheet No. 6060 ($CHCl_3$-MeOH-AcOH, 9 : 1 : 1)

Fig. 1. Ultraviolet absorption spectra of enaminomycins A, B and C in MeOH.

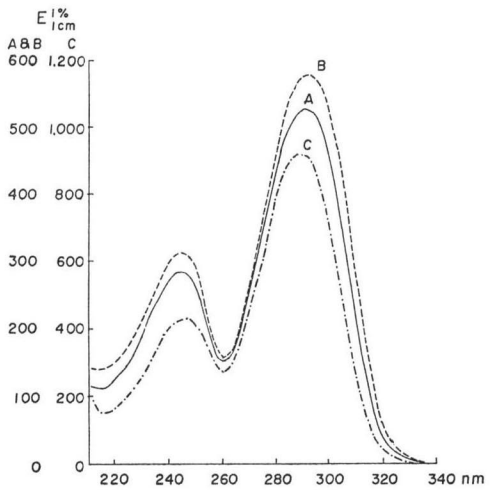

Fig. 2. Infrared absorption spectra of enaminomycins A, B and C (KBr).

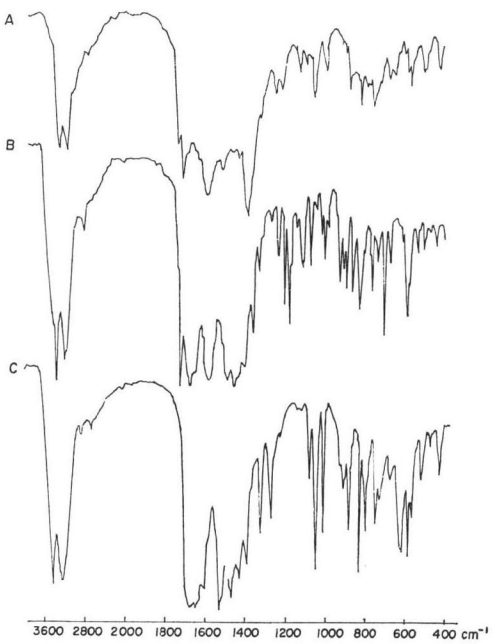

Table 1. The UV, IR and NMR spectra are shown in Figs. 1, 2 and 3, respectively. The UV spectra indicate that they have closely related chromophores, and the NMR spectra indicate the presence of an epoxide group (refer to the signals of AB type protons, J=4.5 Hz). These data strongly suggest that enaminomycins are new members of the

Fig. 3. NMR spectra of enaminomycins A, B and C.

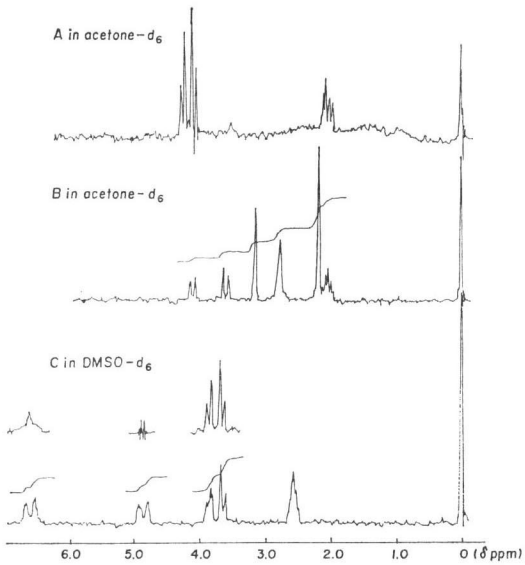

Table 2. Antimicrobial activity of enaminomycins (Paper disc-plate method*).

Enaminomycin	(μg/disc)	Test organism	
		Bacillus subtilis PCI 219	*Proteus vulgaris* OX 19
A	40	25.8 mm	21.5
	20	22.8	18.3
	10	19.4	15.9
B	400	16.8	14.0
	200	12.1	9.0
	100	8.5	0
C	200	25.3	26.2
	100	21.6	22.7
	50	19.4	19.7
A-Me**	800	18.4	18.4
	400	10.8	15.0
	200	0	11.2
B-Me**	800	23.4	24.2
	400	20.8	19.9
	200	17.0	14.1
C-Me**	400	18.9	—
	200	15.4	—
	100	11.1	—

* Toyo paper disc, 8-mm diameter, thick, was used.

** Diazomethane reaction products of enaminomycins.

epoxy quinone family.

Biological Activity

The antimicrobial activity of enaminomycins A, B and C against *Bacillus subtilis* PCI 219 and *Proteus vulgaris* OX19 was determined by the paper disc-plate method, and is expressed in Table 2 in terms of the diameter in millimeters of the inhibition zone. The antimicrobial activities of enaminomycins B and C are respectively one-fifth and one-hundredth that of enaminomycin A against both *B. subtilis* and *P. vulgaris*.

Table 3. Antimicrobial spectrum of enaminomycin A.

Test organism	Medium*	MIC (μg/ml)
Staphylococcus aureus FDA 209P JC-1	1	25
S. aureus 56	1	50
S. aureus 339	1	25
Bacillus subtilis PCI 219	1	25
Micrococcus luteus PCI 1001	1	25
Corynebacterium equi SANK 73460	1	50
Mycobacterium smegmatis ATCC 607	1	>100
Escherichia coli NIHJ JC-2	1	25
E. coli SANK 72075 (CP TC Resistant)**	1	12.5
Proteus vulgaris OX 19	1	12.5
P. morganii SANK 72062	1	12.5
P. mirabilis SANK 71962	1	25
Alcaligenes faecalis SANK 71668	1	12.5
Pseudomonas aeruginosa SANK 73860	1	12.5
P. aeruginosa SC 8753	1	25
Candida albicans YU 1200	2	>100
Penicillium chrysogenum Q176	2	>100
Trichophyton mentagrophytes SANK 11868	2	>100
Pellicularia filamentosa SANK 22272	3	50
Pyricularia oryzae SANK 16975	3	>100
Blastomyces brasiliensis SANK 20567	3	100

* 1: Heart infusion agar
2: SABOURAUD dextrose agar
3: Potato dextrose agar

** Resistant to chloramphenicol and tetracycline.

The reaction products of enaminomycins with diazomethane also showed antibacterial activity. Methylation of enaminomycins A and C decreased the activity by a factor of 80~100 and 80 respectively, but methylation of B increased its activity two-fold.

Table 4. Acute toxicity of enaminomycins A, B and C in mice (ddY, ♂).

Enaminomycin	Route	LD_{50} (mg/kg)
A	i.v.	28.5
B	i.p.	>300
C	i.p.	>300

The minimum inhibitory concentrations (MIC) of enaminomycin A against bacteria, fungi and yeasts were determined by a serial two-fold agar dilution method. The results are shown in Table 3. The medium used for bacteria was heart infusion agar containing 1% glycerol; the medium for yeasts and some fungi, such as *Candida, Saccharomyces, Trichophyton, Aspergillus* and *Penicillium*, was SABOURAUD dextrose agar; and the medium for the other fungi was potato dextrose agar. The MICs were read after one to two days at 37°C for bacteria, and 2~14 days at 27°C for yeasts and fungi. Enaminomycin A was active against Gram-positive and Gram-negative bacteria, especially *Escherichia coli, Pseudomonas aeruginosa* and *Proteus* with MIC value of 12.5 μg/ml, and weakly active against some fungi, such as *Pellicularia filamentosa* and *Blastomyces brasiliensis* at 50~100 μg/ml. Enaminomycins were inactive even at 100 μg/ml against the twelve strains of *Mycoplasma* tested.

The cytostatic effect on L1210 mouse leukemia cells was determined *in vitro* by the method of ISHIWATA *et al.*[2] Viable cells were counted after incubation of the cells in the presence of 0.1 and 1.0 μg/ml of enaminomycin A for 24 hours at 37°C. The mortalities of the cells treated with 0.1 μg/ml and 1.0 μg/ml were 36.8% and 100%, respectively. Enaminomycins B and C had no cytostatic or lethal effect on the cells even at 10 μg/ml.

The acute toxicities of enaminomycins A, B and C are given in Table 4.

Acknowledgements

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