ANTHRACYCLINE METABOLITES FROM *Streptomyces violaceus* A262

II. NEW ANTHRACYCLINE EPELMYCINS PRODUCED BY A BLOCKED MUTANT STRAIN SU2-730

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New anthracycline antibiotics, identified as ε-rhodomycinone glycosides, were isolated from the culture broth of a blocked mutant of β-rhodomycin-producing *Streptomyces violaceus* A262. They were designated as epelmycins A, B, C, D and E, and assayed for their *in vitro* cytotoxicities against murine leukemic L1210 cell culture and the antimicrobial activities in comparison with known anthracycline antibiotics.

In a preceding paper[1], we described the mutational derivation of antibiotic-blocked mutants and variant strains from *Streptomyces violaceus* A262 which produced known diglycosidic β-rhodomycins, A262-1, A262-2, and A262-3. One of these, strain SU2-730, was found to produce new ε-rhodomycinone glycosides in the absence of parental β-rhodomycins. Two families of anthracycline antibiotics with a 10-methoxycarbonyl group are known, the aclacinomycins[2] and the cinerubins (1-hydroxyaclacinomycins)[3]. However, a microbial strain producing either 11-hydroxyaclacinomycins or 1,11-hydroxyaclacinomycins has not been obtained until now.

In this paper, we describe the 11-hydroxyaclacinomycins (ε-rhodomycinone glycosides), designated as epelmycins[4], which were produced by a blocked mutant derived from the β-rhodomycin-producing *S. violaceus* A262 and their biological activities.

Materials and Methods

Microorganisms

The epelmycin-producing strain SU2-730 was isolated from *S. violaceus* A262 as previously described[1], cultivated at 28°C on YS agar slant (yeast extract 0.3%, soluble starch 1.0% and agar 1.5%, pH 7.2) and then stored at 5°C. This strain was deposited as FERM P-8166 in Fermentation Research Institute, Agency of Industrial Science and Technology.

Fermentation

Seed cultures were grown aerobically at 28°C for 48 hours in 500-ml Erlenmeyer flasks containing

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100 ml of a following medium; soluble starch 0.5%, glucose 0.5%, soybean meal 1.0%, yeast extract 0.1%, NaCl 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, pH 7.4, and were used as inoculum (5%) for two 30-liter jar fermenters each containing 15 liters of fermentation medium which contained soluble starch 50 g, soybean meal 30 g, yeast extract 2 g, NaCl 2 g, $CaCO_3$ 2 g, $CuSO_4 \cdot 5H_2O$ 0.01 g, $FeSO_4 \cdot 7H_2O$ 0.0016 g, $ZnSO_4 \cdot 7H_2O$ 0.0032 g, $MnCl_2 \cdot 4H_2O$ 0.013 g in 1,000 ml of tap water, pH 7.0. Fermentation conditions were 15 liters/minute for aeration and 300 rpm for agitation. The cultivation was carried out at 28°C for 130 hours when the mycelial red pigments were produced at a maximum. About 30 liters of the culture broth was prepared for the isolation of purified epelmycins.

Biological Activity

In vitro cytotoxicitiy and inhibition of DNA and RNA syntheses in cells of murine leukemia L1210 were assayed according to the method previously described[5]. Antimicrobial activity was assayed by a standard broth dilution method†.

Qualitative Determination of Aglycone and Sugars by TLC

Epelmycin in 0.1 N HCl (10 mg/ml) was heated at 85°C for 30 minutes in a water bath. The aglycone thus obtained was extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness *in vacuo* and the red pigment residue was then purified by preparative TLC on Silica gel plate PF_{254} (E. Merck) using a developing solvent of $CHCl_3$ - MeOH (20 : 1). The purified aglycone was subjected to TLC on Silica gel F_{254} (E. Merck) using a developing solvent of $CHCl_3$ - MeOH (20 : 1).

Alternatively, the aqueous layer containing sugar components was neutralized by adding silver carbonate with a small amount of charcoal and centrifuged. The supernatant fluid was concentrated *in vacuo* and subjected to TLC on Silica gel plate F_{254} using a developing solvent of BuOH - acetic acid - H_2O (4 : 1 : 1). The sugar spots were detected by spraying with *p*-anisaldehyde - H_2SO_4 (each 5%) in 90% EtOH and heating at 90°C. Aclarubicin, aclacinomycin B and MA144-N1[2] were also hydrolyzed in the same manner and the aqueous layers were used as a source of authentic sugars including L-rhodosamine, 2-deoxy-L-fucose, L-rhodinose, L-cinerulose A and L-cinerulose B, located on a TLC plate with Rf values of 0.12, 0.56, 0.71, 0.82 and 0.80, respectively.

Mild Acid Hydrolysis

Epelmycin was partially hydrolized by standing a solution of 1 mg/ml in 0.1 N HCl at room temperature for 1 hour. After being alkalified to pH 8.0 with 0.1 N NaOH, the hydrolysate was extracted with $CHCl_3$ and the organic layer was evaporated *in vacuo* to dryness. The red pigment residue was subjected to TLC on Silica gel plate F_{254} using a developing solvent of $CHCl_3$ - MeOH (15 : 1).

HPLC Analysis

To check the purity of products from the isolation and purification processes, HPLC was performed on a Hitachi 655 liquid chromatographic apparatus with a reverse phase analytical column, μBondapak phenyl (3.9 × 300 mm) (Waters Associates). Acetonitrile - 0.04 M ammonium formate (pH 3.0, 60 : 40) was used as a mobile phase and run at a flow rate of 1.5 ml/minute. Samples were dissolved in the mobile phase and 10 μl samples injected. Detection was at 254 nm using a UV detector (UVILOG-5IIIA, Oyo-Bunko Kiki Co., Ltd.).

General

MP's were determined on a Kofler hotstage microscope. UV spectra were determined on a Hitachi EPS 3T and IR spectra (KBr pellet) on a Hitachi EPI-GS spectrophotometer. ^1H and ^{13}C NMR were recorded with a Jeol GX-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are expressed in δ values (ppm) with TMS as an internal reference and coupling constants are given in J (Hz). Mass spectra were recorded with a Hitachi M-80H spectrometer. Specific rotations were determined on a Jasco DIP-181 Digital Polarimeter.

† National Committee for Clinical Laboratory Standards: Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards, NCCLS, Villanova, 1983.

Results and Discussion

Isolation and Purification of Epelmycins

The fermentation broth (30 liters) was collected from two runs of a 30-liter jar fermentations and filtered, and the anthracycline products were extracted from the mycelial cake with a total of 10 liters of acetone. The acetone extract was evaporated *in vacuo* to about 2 liters. The concentrate was extracted with 3 liters of $CHCl_3$ after being adjusted to pH 8.0 with 4 N NaOH. The $CHCl_3$ extract was evaporated *in vacuo* to a small volume and an excess of *n*-hexane was added to precipitate the crude mixture of anthracycline products. This isolation procedure provided about 7.6 g of red crude powder, which could be resolved into five red components by chromatography on Silica gel plate F_{254} using a solvent mixture of $CHCl_3$ - MeOH (8 : 1). The crude powder was chromatographed on a column of silica gel (Wakogel C-200) which was developed with solvent mixtures of $CHCl_3$ - MeOH (in the order, stepwise, 100 : 1, 100 : 3 and 100 : 5) which eluted epelmycins in the following order B, A, E, C and D. Each compound was further purified by preparative TLC using a solvent mixture of $CHCl_3$ - MeOH - aqueous NH_3 (120 : 10 : 0.2). Each epelmycin band on the TLC plate was removed and extracted with $CHCl_3$ - MeOH (8 : 1). The extract was evaporated to dryness. The antibiotics recovered were dissolved in 20 ml of 0.1 M acetate buffer (pH 3.5) and washed with 10 ml of toluene. The aqueous layer was adjusted to pH 7.5 by addition of saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and concentrated after being dried over Na_2SO_4. An excess of *n*-hexane was added to precipitate red powder. Purified epelmycins A, B, C, D and E were obtained with yields of 35 mg, 149 mg, 44 mg, 27 mg and 26 mg, respectively. The purity of these compounds was in excess of 95% as determined by HPLC, where epelmycins A, B, C, D and E had retention times of 16.0, 11.3, 4.9, 6.6 and 8.6 minutes, respectively.

Epelmycins are soluble in acetone, methanol, ethanol, chloroform, ethyl acetate, benzene and acidic water, but insoluble or slightly soluble in ether, hexane and water.

Structural Determination of Epelmycins

The structural determination of epelmycins was mainly carried out by FD-MS, ^1H and ^{13}C NMR

Table 1. Physico-chemical properties of epelmycins.

	Epelmycin A	Epelmycin B	Epelmycin C	Epelmycin D	Epelmycin E
Molecular formula	$C_{42}H_{53}NO_{15}$	$C_{42}H_{51}NO_{16}$	$C_{36}H_{45}NO_{14}$	$C_{30}H_{35}NO_{11}$	$C_{42}H_{53}NO_{16}$
FD-MS (*m/z*)	812 $(M+H)^+$	826 $(M+H)^+$	716 $(M+H)^+$	585 M^+	827 M^+
MP (°C, dec)	157~159	175~178	164~167	152~154	160~163
$[\alpha]_D^{23}$ (*c* 0.02, $CHCl_3$)	+248°	+69°	+275°	+520°	+104°
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	209 (270), 235 (538), 255 (314), 294 (103), 493 (181), 525 (sh, 121), 584 (sh, 20)	207 (254), 235 (548), 254 (328), 293 (107), 492 (189), 525 (sh, 129), 587 (sh, 22)	208 (300), 235 (621), 252 (412), 295 (122), 494 (200), 528 (161), 585 (86)	207 (289), 235 (655), 255 (405), 293 (135), 493 (242), 526 (sh, 157), 581 (sh, 15)	207 (277), 235 (594), 254 (357), 294 (118), 492 (206), 527 (sh, 139), 581 (sh, 22)
IR ν_{max} (KBr) cm^{-1}	3450, 2930, 1730, 1600, 1400, 1290, 1200, 1120, 1000	3420, 2930, 1730, 1600, 1400, 1290, 1200, 1120, 1010	3450, 2950, 1730, 1600, 1400, 1290, 1200, 1120, 1010	3420, 2920, 1730, 1600, 1400, 1290, 1200, 1120, 1010, 980	3430, 2930, 1730, 1600, 1400, 1290, 1200, 1120, 1010
Rf value[a]	0.56	0.67	0.22	0.14	0.53

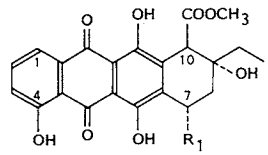
[a] The values were obtained on silica gel TLC, $CHCl_3$ - MeOH (8 : 1).

Table 2. TLC analysis of sugars of epelmycins.

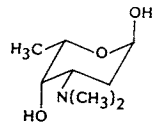
Compound	Spots on TLC	
	Rf value[a] (color[b])	Sugar detected
Epelmycin A	0.12 (Sky blue)	Rhodosamine
	0.71 (Green)	Rhodinose
	0.82 (Greenish blue)	Cinerulose A
Epelmycin B	0.12 (Sky blue)	Rhodosamine
	0.56 (Grayish blue)	2-Deoxyfucose
	0.80 (Greenish blue)	Cinerulose B
Epelmycin C	0.12 (Sky blue)	Rhodosamine
	0.56 (Grayish blue)	2-Deoxyfucose
Epelmycin D	0.12 (Sky blue)	Rhodosamine
Epelmycin E	0.12 (Sky blue)	Rhodosamine
	0.56 (Grayish blue)	2-Deoxyfucose
	0.82 (Greenish blue)	Cinerulose A

[a] BuOH - acetic acid - H_2O (4 : 1 : 1).
[b] Visualization was carried out with *p*-anisaldehyde.

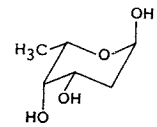
Fig. 1. Structures of epelmycins.



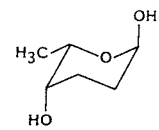
Epelmycins	R_1
A	RN-R-CinA
B	RN-dF-CinB
C	RN-dF
D	RN
E	RN-dF-CinA



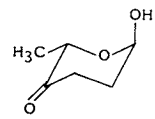
Rhodosamine (RN)



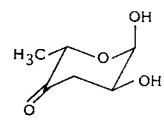
2-Deoxyfucose (dF)



Rhodinose (R)



Cinerulose A (CinA)



Cinerulose B (CinB)

analyses and TLC analysis of the sugar components obtained by acid hydrolysis.

The physico-chemical properties of epelmycins are shown in Table 1. The UV and visible light absorption spectra of all epelmycins in 90% MeOH solution were similar to each other with a resemblance to ε-rhodomycinone. Epelmycins are pH indicators having a red color at neutral and acidic pH ranges while they are purple at alkaline pH. The IR absorption spectra of epelmycins indicated the presence of an ester carbonyl (1730 cm^{-1}) and an hydrogen bonded carbonyl (1600 cm^{-1}) which are characteristic of anthracyclines.

On total acid hydrolysis followed by TLC analysis, epelmycins gave a red aglycone and the corresponding sugar components. The aglycone was identified as ε-rhodomycinone from its UV, mass (*m/z* 428 M^+) and ^1H and ^{13}C NMR spectra, while the sugars were found to be rhodosamine (RN), 2-deoxyfucose (dF), cinerulose A (CinA) and cinerulose B (CinB) by a direct comparison with authentic samples (from aclacinomycins) on TLC. Results of sugar analysis are shown in Table 2.

On mild acid hydrolysis, epelmycins A, B and C gave D while E yielded C and D. Epelmycin D was composed of ε-rhodomycinone and a sugar, RN and was identified as 7-*O*-rhodosaminyl-ε-rhodomycinone consistent with a molecular formula of $C_{30}H_{35}NO_{11}$ (*m/z* 585 M^+) and its ^1H and ^{13}C NMR spectra. These results indicate that epelmycins A, B, C and E have one or two sugars added to epelmycin D. By analogy with the sugar moiety of aclacinomycins it was inferred that epelmycins A, B, C and E had a sugar chain of RN-R-CinA, RN-dF-CinB, RN-dF and RN-dF-CinA linked to ε-rhodomycinone, respectively. These data suggest the structures of the different epelmycins shown in Fig. 1.

The chemical shift assignments for the ^1H and ^{13}C NMR spectra of epelmycins were carried out by means of pulse technique, DEPT, ^1H-^1H and ^1H-^{13}C COSY, and are shown in Tables 3 and 4, respectively. In ^{13}C NMR spectra, there is a difference in the chemical shift of C-7 between ε-rhodomycinone and the corresponding aglycone moiety in epelmycin molecule. The chemical shift of C-7 was 61.8 ppm for

Table 3. ^{1}H NMR chemical shifts of epelmycins and ε-rhodomycinone (ε-RMN).

Proton	Epelmycin A	Epelmycin B	Epelmycin C	Epelmycin D	Epelmycin E	ε-RMN
1-H	7.88 dd (8, 1)	7.85 d (8)	7.84 d (8)	7.86 d (8)	7.85 d (8)	7.72 d (8)
2-H	7.70 t (8)	7.68 t (8)	7.69 t (8)	7.70 t (8)	7.69 t (8)	7.64 t (8)
3-H	7.32 dd (8, 1)	7.28 d (8)	7.30 d (8)	7.30 d (8)	7.30 dd (8, 1)	7.25 d (8)
4-OH	13.54 s	13.50 br	13.48 br	13.48 br	13.50 br s	—
6-OH	12.88 br	12.85 br	12.85 br	12.87 br	12.87 br	—
11-OH	12.18 s	12.13 br	12.11 br	12.13 br	12.14 br s	—
7-H	5.25 br	5.23 d (4)	5.23 br d	5.23 br d	5.24 bd	5.26 d (3)
8-Ha	2.33 d (15)	2.33 d (15)	2.35 d (15)	2.35 d (15)	2.34 d (15)	2.29 d (15)
8-Hb	2.22 dd (15, 4)	2.21 dd (15, 4)	2.21 dd (15, 4)	2.21 dd (15, 4)	2.23 dd (15, 4)	2.18 dd (15, 5)
9-OH	4.63 s	4.59 s	4.62 s	4.62 s	4.62 s	—
10-H	4.28 s	4.27 s	4.29 s	4.29 s	4.28 s	4.23 s
13-Ha	1.8 m	1.8 m	1.8 m	1.8 m	1.8 m	1.81 m (7)
13-Hb	1.4 m	1.4 m	1.4 m	1.4 m	1.4 m	1.54 m (7)
14-CH_3	1.12 t (7)	1.12 t (7)	1.13 t (7)	1.13 t (7)	1.12 t (7)	1.14 t (7)
16-OCH_3	3.72 s	3.72 s	3.72 s	3.72 s	3.72 s	3.72 s
1′-H	5.54 d (4)	5.48 br s	5.53 br s	5.52 br s	5.52 br s	—
2′-CH_2	1.8	1.8	1.8	1.8	1.8	—
3′-H	1.97 t (8)	2.07 t (8)	2.1	2.2	2.15	—
4′-H	3.78 br s	3.76 br s	3.77 br s	3.70 br s	3.74 br s	—
5′-H	4.02 q (7)	4.01 q (7)	4.03 q (7)	4.07 q (7)	4.02 q (7)	—
6′-CH_3	1.27 d (7)	1.27 d (7)	1.29 d (7)	1.39 d (7)	1.28 d (7)	—
3′-$N(CH_3)_2$	2.19 s	2.15 s	2.18 s	2.21 s	2.18 s	—
1″-H	4.95 br d	5.10 br d	5.01 br d	—	5.04 br d	—
2″-Ha	2.3	2.45 dt (12, 4)	2.1	—	2.10 dd (12, 5)	—
2″-Hb	1.8	1.92 dd (12, 5)	1.8	—	1.8	—
3″-H	—	4.33 dt (12, 4)	4.12 dt (12, 4)	—	4.12 br	—
3″-CH_2	2.1 m	—	—	—	—	—
4″-H	3.58 br s	4.01	3.64 br s	—	3.67 br s	—
5″-H	4.48 q (7)	4.66 q (7)	4.55 q (7)	—	4.56 q (7)	—
6″-CH_3	1.13 d (7)	1.23 d (7)	1.22 d (7)	—	1.17 d (7)	—
1‴-H	5.06 t (5)	5.19 br d	—	—	5.08 t (6)	—
2‴-H	—	4.37 m	—	—	—	—
2‴-Ha	2.4 m	—	—	—	2.4 m	—
2‴-Hb	2.1	—	—	—	2.1	—
3‴-CH_2	2.5 m	2.59	—	—	2.46	—
5‴-H	4.34 q (7)	4.79 q (7)	—	—	4.49 q (7)	—
6‴-CH_3	1.28 d (7)	1.33 d (7)	—	—	1.32 d (7)	—

Values were obtained in ppm (δ) (J=Hz) from $CDCl_3$ solutions (epelmycins) or $CDCl_3$-CD_3OD (10:1) solution (ε-rhodomycinone) containing TMS as an internal reference at 400 MHz.

ε-rhodomycinone and 70.6~71.1 ppm for epelmycins. This down-field shift is attributable to a 7-*O*-sugar linkage as seen with other anthracyclines[6]. The chemical shift assignments of C-5′, C-4″ and C-5″ of epelmycin B and C-5′, C-6′, C-1″, C-5″, C-6″ and C-1‴ of epelmycin E were different from those of aclacinomycin B and aclarubicin which have the same sugar chains, respectively, but others were almost identical. In epelmycins B and E, further elucidation of assignments were carried out by the correlation *via* long range coupling (COLOC) experiment as shown in Fig. 2. In particular the long range coupling of C-3″ with 2‴-H in epelmycin B supported the presence of an ether bond between dF and CinB. The small coupling constants (J=~4 Hz) of all anomeric protons in epelmycins indicate that the configurations of glycosidic bonds are all α.

From all these findings the structures of epelmycins were determined as illustrated in Fig. 1. Epelmycin E was identical to CG5 which was obtained by microbial transformation[7]. This is the first case of a

Table 4. ^{13}C NMR chemical shifts of epelmycins and ε-rhodomycinone (ε-RMN).

Carbon	Epelmycin A	Epelmycin B	Epelmycin C	Epelmycin D	Epelmycin E	ε-RMN
1	119.6	119.6	119.6	119.6	119.6	119.4
2	137.0	137.0	137.0	137.0	137.0	137.7
3	124.8	124.8	124.8	124.8	124.8	124.6
4	162.7	162.6	162.6	162.7	162.6	162.1
4a	116.2	116.1	116.1	116.1	116.1	115.6
5	190.9	190.8	190.7	190.8	190.8	190.3
5a	111.6	111.6	111.6	111.6	111.6	111.1
6	157.0	156.9	(157.0)	156.9	156.9	(155.9)
6a	136.2	136.0	136.1	136.0	136.1	134.4
7	70.6	70.7	70.7	71.1	70.7	61.8
8	33.4	33.3	33.4	33.3	33.4	34.2
9	71.3	71.2	71.2	71.3	71.2	71.5
10	52.2	52.2	52.2	52.2	52.2	52.3
10a	135.5	135.5	135.5	135.5	135.5	136.9
11	157.0	156.9	(156.9)	156.9	157.0	(156.2)
11a	111.3	111.2	111.2	111.3	111.2	110.9
12	186.2	186.1	186.1	186.2	186.1	185.7
12a	133.6	133.4	133.5	133.4	133.5	133.0
13	32.4	32.4	32.4	32.4	32.4	32.4
14	6.8	6.8	6.8	6.8	6.8	6.5
15	171.4	171.4	171.4	171.3	171.4	171.3
16	52.4	52.4	52.4	52.4	52.3	51.5
1′	101.8	101.7	101.7	101.5	101.8	—
2′	29.4	29.3	29.3	29.4	29.3	—
3′	61.7	61.6	61.6	59.4	61.6	—
4′	73.9	74.2	74.3	66.0	74.1	—
5′	68.7	68.4	68.4	66.6	68.5	—
6′	17.9	17.8	17.9	17.0	17.9	—
3′-$N(CH_3)_2$	43.3	43.2	43.3	42.0	43.3	—
1″	(98.7)	99.1	99.5	—	99.4	—
2″	24.7	27.0	33.1	—	34.4	—
3″	24.9	67.3	65.9	—	65.4	—
4″	75.6	66.9	71.6	—	83.0	—
5″	66.7	65.3	66.2	—	66.8	—
6″	17.1	16.0	16.7	—	17.0	—
1‴	(99.0)	91.5	—	—	100.1	—
2‴	28.7	63.0	—	—	27.7	—
3‴	33.7	39.8	—	—	33.5	—
4‴	211.0	208.1	—	—	209.9	—
5‴	71.2	77.9	—	—	71.8	—
6‴	14.9	16.2	—	—	14.8	—

Values were obtained in ppm (δ) from $CDCl_3$ solutions (epelmycins) or $CDCl_3$-CD_3OD (10:1) solution (ε-RMN) containing TMS as an internal reference at 100 MHz.

Similar values in parentheses may be interchanged.

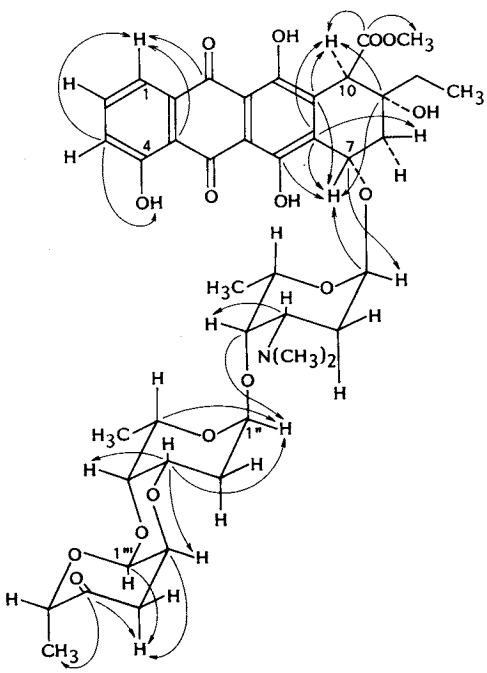
microbial strain producing ε-rhodomycinone glycosides which contain rhodosamine, no such blocked mutant or variant strain of the aclacinomycin-producer *Streptomyces galilaeus* MA144-M1 has been obtained[8]. Epelmycins B, C, D and E have the same sugar chains in their structure as aclacinomycins B, S, T and aclarubicin, respectively.

Biological Activity

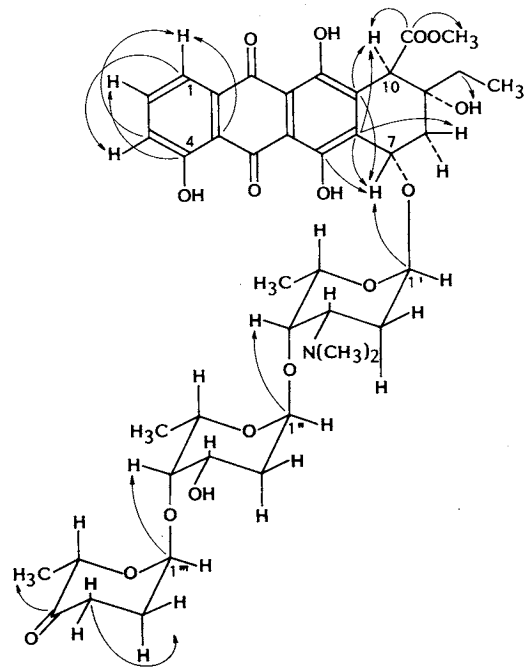
The antimicrobial activities of epelmycins are shown in Table 5. MIC was estimated by the broth-dilution method. The epelmycins exhibited antimicrobial activity against all of the Gram-positive

Fig. 2. COLOC experiment of epelmycin B and epelmycin E.

Arrows indicate ^{1}H-^{13}C long range couplings.



Epelmycin B



Epelmycin E

Table 5. Antimicrobial activity of epelmycins (EPM) A, B, C, D, E, aclarubicin (ACM-A) and doxorubicin (ADM).

Microorganism	MIC (μg/ml)						
	EPM-A	EPM-B	EPM-C	EPM-D	EPM-E	ACM-A	ADM
Staphylococcus aureus FDA 209P	3.13	0.78	3.13	3.13	3.13	6.25	6.25
Bacillus subtilis ATCC 6633	0.78	0.39	0.78	0.78	0.78	3.13	3.13
B. cereus ATCC 9634	0.20	0.10	0.20	0.10	0.20	0.39	1.57
B. megaterium NRRL B-938	0.39	0.20	0.39	0.78	0.39	1.57	3.13
Micrococcus luteus ATCC 9341	0.78	0.39	0.78	0.78	0.78	1.57	1.57
M. flavus	0.78	0.20	0.78	0.78	0.78	0.78	3.13
Corynebacterium bovis 1810	0.78	0.39	0.39	0.39	0.78	0.78	5.00
Mycobacterium smegmatis ATCC 607	1.57	0.78	0.39	0.05	0.78	0.78	1.57
Pseudomonas fluorescens NIHJ B-25	0.78	0.05	0.39	0.39	0.78	0.78	3.13
Candida albicans IAM 4905	0.78	1.57	1.57	6.25	1.57	2.50	3.13

and Gram-negative bacteria tested and *Candida albicans*. Epelmycins were more active than aclarubicin and doxorubicin.

The cytotoxic activities of epelmycins against leukemic L1210 cell culture were examined under continuous exposure and are shown in Table 6. The epelmycins were more potent than the aclacinomycins, doxorubicin and daunorubicin. The epelmycins exhibited a preferential inhibition of RNA synthesis

Table 6. Inhibitory activity of epelmycins and other anthracyclines on the growth and nucleic acid synthesis of murine leukemic L1210 cell culture.

Compound	IC_{50} (μg/ml)			DNA/ RNA	Compound	IC_{50} (μg/ml)			DNA/ RNA
	Growth	DNA synthesis	RNA synthesis			Growth	DNA synthesis	RNA synthesis	
Epelmycin A	0.004	0.72	0.09	8.0	Aclacinomycin B	0.01	0.92	0.11	8.4
Epelmycin B	0.001	0.60	0.16	3.8	MA144 S1	0.025	0.39	0.08	4.9
Epelmycin C	0.004	0.80	0.20	4.0	MA144 T1	0.047	0.72	0.31	2.3
Epelmycin D	0.006	0.95	0.23	4.1	Doxorubicin	0.02	1.40	0.55	2.5
Epelmycin E	0.008	0.80	0.15	5.3	Daunorubicin	0.02	0.42	0.16	2.6
Aclarubicin	0.01	0.65	0.085	7.6					

In the inhibition test for nucleic acid synthesis, the drugs were exposed for 60 minutes to L1210 cell culture (8×10^5 cells/ml) with supplemented ^{14}C-labeled uridine or thymidine (0.05 μCi/ml), and the incorporation of the radioisotopes into acid insoluble material was measured. For the growth inhibition test, the drugs were exposed for 48 hours to L1210 cell culture (5×10^4 cells/ml) and the viable cells were counted by coulter counter.

IC_{50} is expressed as a drug concentration required to inhibit by a 50% control of the growth, and DNA and RNA syntheses of cultured L1210 cells.

compared with DNA synthesis like aclarubicin. Therefore, the epelmycins seem to belong to Class II anthracyclines in their mode of biochemical action[9].

Antitumor effects *in vivo* of epelmycins were tested in mice bearing leukemia L1210 by daily ip administration from day 1 to 10. Epelmycins B, C, D and E had a maximum antitumor activity of 119, 179, 141 and 154% (T/C) at an optimum dose of 125, 50, 100 and 25 μg/mouse/day, respectively. Under the same conditions aclarubicin and doxorubicin had a T/C of 180 and 290% at an optimum dose of 75 and 25 μg/mouse/day, respectively.

The epelmycins exhibited stronger growth-inhibition activity against both microorganisms and cultured leukemic L1210 cells than aclacinomycins. This fact suggests that a hydroxyl group at C-11 plays an important role in the biological activity.

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