NOTES

DIFFERENTIAL ANTIMICROBIAL ACTIVITIES OF ANTHRACYCLINE ANTIBIOTICS ON REC⁻ *BACILLUS SUBTILIS*

AKIHIRO YOSHIMOTO, TOSHIKAZU OKI and TAIJI INUI

Central Research Laboratories
Sanraku-Ocean Co., Ltd.
Fujisawa, Kanagawa, Japan

(Received for publication May 23, 1977)

Adriamycin and daunomycin have been reported to show mutagenic action in a test system for frameshift mutagens or for excision repair using mutants of *Salmonella typhimurium*.[1,2] These compounds have also been reported to induce mammary and renal tumors in rats[3] as well to cause malignant transformation and mutation in mammalian cell system *in vitro*.[4]

Rec⁻ mutants have been reported to be much more sensitive to mutagenic and carcinogenic compounds than rec⁺ strains. We have therefore examined the activities of various new anthracycline antibiotics[5~8] in inhibiting *Bacillus subtilis* strains H17A (rec⁺, try⁻) and M45T (rec⁻, arg⁻).[9~11]

The above strains were kindly provided by Dr. T. KADA, National Institute of Genetics, Mishima. Instead of the streak test method employed by KADA *et al.*,[9] a cup diffusion method was used for the quantitative assay of bacterial growth. Commercial bouillon (Kyokuto) supplemented with 20 μg/ml each of L-tryptophan and L-arginine was used as the medium. Twenty milliliters of a basal layer containing 1.5% agar were solidified in a petri dish (9 cm in diameter), and 4 ml of the melted top layer agar (1.5% Difco agar) containing either rec⁻ or rec⁺ cells (one drop of overnight-shaking culture at 28°C to 50 ml of the top layer) were overlaid. After permitting the diffusion of the sample into the agar plate at 4°C for 2 hours, the plates were incubated at 28°C for 20 hours and the diameter of the growth inhibition zone was measured. Aclacinomycin A and the others were dissolved at a concentration of 8 mg/ml in 0.5 ml of methanol containing one drop of 0.05 N HCl and the solution was then diluted with 1% potassium phosphate buffer, pH 6.0. Chromomycin A_3 (Toyomycin, Takeda Pharm. Ind. Co.), citrimycin,[12] carminomycin I and the hydrochloride salts of adriamycin (Kyowa Hakko Ind. Co.) and daunomycin (Meiji Seika Co.) were dissolved in the same manner. The aglycones and the acridine dye were dissolved at 2 mg/ml in methanol and diluted with a mixture of dimethylsulfoxide - 1% potassium phosphate (1 : 1).

Fig. 1 shows the dose-response curves of aclacinomycin A and adriamycin using isogenic strains of *Bacillus subtilis* with or without recombination-repair system. Aclacinomycin A showed only a slight differential antimicrobial effect on the pair of strains, while adriamycin showed 4-times stronger inhibition against rec⁻ strain than against rec⁺ strain. This suggests that aclacinomycin A has considerably less differential activity than adriamycin.

The results of the *rec* assay for 20 new anth-

Fig. 1. Antimicrobial effect of aclacinomycin A and adriamycin on *Bacillus subtilis*.

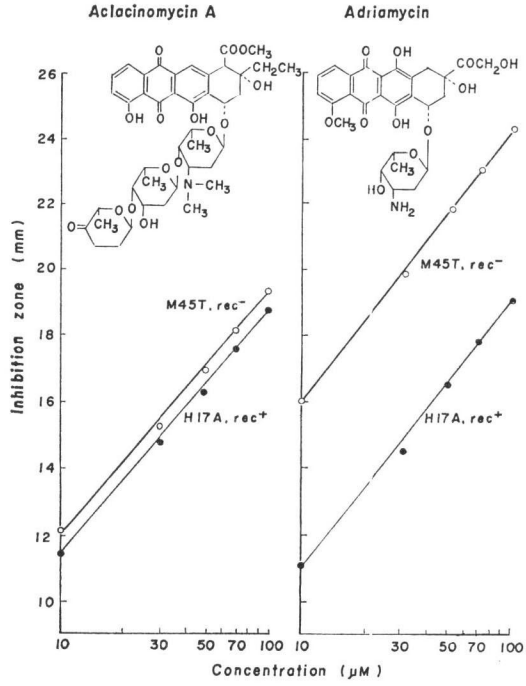


Table 1. Antimicrobial activity of anthracycline antibiotics against *Bacillus subtilis* strain H17A, rec$^+$ and M45T, rec$^-$

Compounds	Structure*2		Antimicrobial activity*1		Potency index rec$^+$/rec$^-$
	Aglycones	Sugars	H17A (rec$^+$)	M45T (rec$^-$)	
Aclacinomycin A	Aklavinone	Ra–DF–LCin A	32	27	1.2
〃 B	〃	Ra–DF–LCin B	36	30	1.2
MA144 G1	〃	Ra–DF–DCin A	41	33	1.2
〃 M1	〃	Ra–DF–Ami	38	32	1.2
〃 N1	〃	Ra–DF–Rho	39	32	1.2
〃 U1	〃	Ra–DF–DF	32	14	2.3
〃 S1	〃	Ra–DF	28	14	2.0
1-Deoxypyrromycin	〃	Ra	150	84	1.8
Cinerubin A	ε-Pyrromycinone	Ra–DF–LCin A	20	16	1.3
Rhodirubin A	〃	Ra–DF–Rho	13	11	1.2
〃 B	〃	Ra–Rho–Rho	34	22	1.5
〃 D	〃	Ra–DF	14	12	1.2
Pyrromycin	〃	Ra	120	56	2.1
Baumycin A1	Daunomycinone	Da–X1	47	12	3.9
〃 A2	〃	〃	88	22	4.0
〃 B2	〃	Da–X2	910	215	4.2
Daunomycin	〃	Da	38	9	4.2
Adriamycin	Adriamycinone	Da	33	8	4.1
Carminomycin I	Carminomycinone	Da	50	14	3.6
Chromomycin A2			16	16	1.0
Citrimycin A			14	15	0.9
〃 L			10	11	0.9
2,8-diamino-10-methyl acridine			13	12	1.1

*1 Concentration (μM) that gives a 15-mm inhibition zone.

*2 Sugars; Ra; L-rhodosamine, Da; L-daunosamine, Cin; cinerulose, Ami; L-amicetose, Rho; L-rhodinose, DF; 2-deoxy-L-fucose

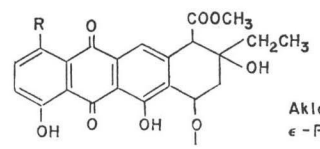
X1; –CH–O–CH–CH$_3$ (CH$_2$; CH$_2$OH; CHOH; CH$_3$)

X2; –CH–O–CH–CH$_3$ (CH$_2$; COOH; CHOH; CH$_3$)

racycline antibiotics are summarized in Table 1 in comparison with adriamycin, daunomycin, carminomycin, some other antitumor antibiotics and an acridine dye, wherein the potency index of the test compounds was expressed by the rec$^+$/rec$^-$ ratio of concentrations (μM) which produced a 15-mm growth inhibition zone against rec$^-$ and rec$^+$ strains. The newly isolated anthracycline antibiotics tested are classified into the following three groups on the basis of their aglycones: aklavinone group,[5] ε-pyrromycinone (1-hydroxyaklavinone) group[7] and daunomycinone-adriamycinone group.[8]

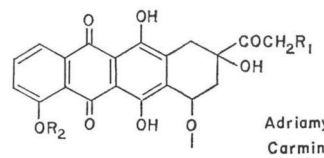
Most new anthracycline glycosides containing aklavinone or ε-pyrromycinone, an acridine dye, chromomycin A$_3$ and citrimycin showed little

Aglycone:



Aklavinone: R = H

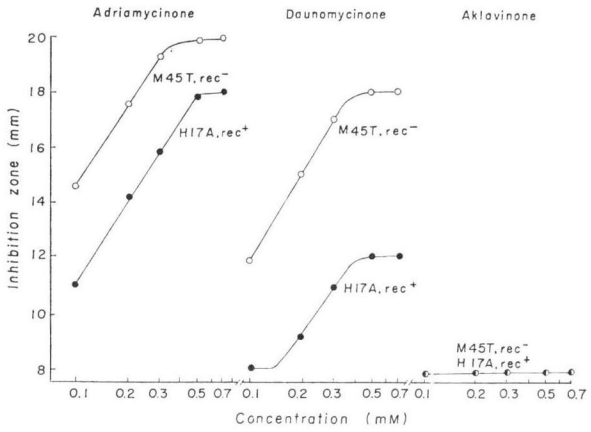
ε-Pyrromycinone: R = OH



	R_1	R_2
Adriamycinone:	OH	CH_3
Carminomycinone:	H	H
Daunomycinone:	H	CH_3

effect on the *rec* assay system, and their indices were as low as 1.2. Some exceptions were observed with MA144 U1, MA144 S1, 1-deoxypyrromycin and pyrromycin which had indices around 2. In contrast, daunomycin, carminomycin I and adriamycin showed strong positive indices which were around 4. Baumycins A1, A2 and B2, which contained daunomycinone as the aglycone, displayed results similar to daunomycin. The results may be summarized that the activity of anthracycline glycosides on the *rec*$^-$ system is influenced by the aglycone structures. We have therefore studied the effect of aglycones as shown in Fig. 2. Adriamycinone and daunomycinone still retain the effect on the *rec* assay, although their antimicrobial activities decreased to less than one-tenth those of the corresponding glycosides. However, aklavinone and 7-deoxyaklavinone showed no inhibitory effect on either pair of strains.

Fig. 2. Antimicrobial effect of adriamycinone, daunomycinone and aklavinone on *Bacillus subtilis*.



Acknowledgement

We would like to express our thanks to Dr. H. UMEZAWA, Emeritus Professor of Tokyo University, and Dr. T. TAKEUCHI, Institute of Microbial Chemistry, Tokyo, for their keen discussions and criticism.

References

1) McCANN, T.; E. CHOI, E. YAMASAKI & B. N. AMES: Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A. 72: 5135~5139, 1975
2) PANI, B.; C. MONTI-BRAGADIN & L. SAMER: Effect of excision repair system on antibacterial and mutagenic activity of daunomycin and other intercalating agents in *Salmonella typhimurium*. Experientia 31: 787~789, 1975
3) BERTAZZOLI, C.; T. CHIELI & E. SOLCIA: Different incidence of breast carcinomas or fibrosdenomas in daunomycin or adriamycin treated rats. Experientia 27: 1209~1210, 1971
4) PRICE, P. J.; W. A. SUK & P. C. SKEEN: Transforming potential of the anticancer drug adriamycin. Science 187: 1200~1201, 1975
5) OKI, T.; Y. MATSUZAWA, A. YOSHIMOTO, K. NUMATA, I. KITAMURA, S. HORI, A. TAKAMATSU, H. UMEZAWA, M. ISHIZUKA, H. NAGANAWA, M. HAMADA & T. TAKEUCHI: New antitumor antibiotics, aclacinomycins A and B. J. Antibiotics 28: 830~834, 1975
6) OKI, T.; N. SHIBAMOTO, Y. MATSUZAWA, T. OGASAWARA, A. YOSHIMOTO, I. KITAMURA, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Production of nineteen anthracyclic compounds by *Streptomyces galilaeus* MA144-M1. J. Antibiotics 30: 683~687, 1977
7) KITAMURA, I.; N. SHIBAMOTO, T. OKI, I. INUI, H. NAGANAWA, M. ISHIZUKA, T. MASUDA, T. TAKEUCHI & H. UMEZAWA: New anthracycline antibiotics, rhodirubins. J. Antibiotics 30: 616~618, 1977
8) KOMIYAMA, T.; Y. MATSUZAWA, T. OKI, T. INUI, Y. TAKAHASHI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Baumycins, new antitumor antibiotics related to daunomycin. J. Antibiotics 30: 619~621, 1977
9) KADA, T.; K. TUKIKAWA & Y. SADAIE: *In vitro* and host-mediated "rec-assay" procedures for screening chemical mutagens, and phloxine, a mutagenic red dye detected. Mutation Res. 16: 165~174, 1972
10) KADA, T.; M. MORIYA & Y. SHIRASU: Screening of pesticides for DNA interactions by "rec-assay" and mutagenesis testing, and frameshift mutagens detected. Mutation Res. 26: 243~248, 1974
11) SADAIE, Y. & T. KADA: Recombination-deficient mutants of *Bacillus subtilis*. J. Bacteriol. 125: 489~500, 1976
12) OKI, T.; Y. MATSUZAWA, K. NUMATA & A. TAKAMATSU: SR1768, an antitumor antibiotic closely related to chromomycin. J. Antibiotics 26: 701~704, 1973