TALLYSOMYCIN*, A NEW ANTITUMOR ANTIBIOTIC COMPLEX RELATED TO BLEOMYCIN

IV. NEW BIOSYNTHETIC DERIVATIVES OF TALLYSOMYCIN

TAKEO MIYAKI, OSAMU TENMYO, KEI-ICHI NUMATA, KIYOSHI MATSUMOTO, HARUAKI YAMAMOTO, YŪJI NISHIYAMA, MASARU OHBAYASHI, HIDEYO IMANISHI, MASATAKA KONISHI and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd., Meguro, Tokyo, Japan

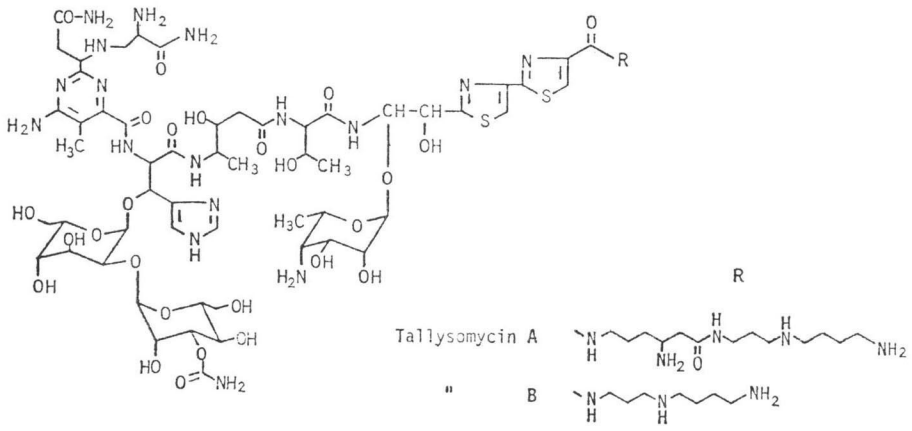
(Received for publication March 19, 1981)

A series of new biosynthetic derivatives of tallysomycins A and B were obtained by precursor amine-feeding fermentation. Certain diamines were incorporated into tallysomycins as the terminal amine moiety affording two classes of biosynthetic derivatives, with or without the β-lysine moiety in the subterminal position. Among 21 derivatives prepared, tallysomycin S_{10b} was selected for further studies in view of its favorable therapeutic indices.

Tallysomycins A and B[1] are glycopeptide antibiotics isolated from fermentation broths of *Streptoalloteichus hindustanus* strain No. E465-94, ATCC 31158.[2] The structures of tallysomycins A and B were initially determined by KONISHI *et al.*[3] and later revised as shown in Fig. 1 according to the structural revision of bleomycin.[4] The antitumor activity of tallysomycin was reported by IMANISHI *et al.*[5] and BRADNER *et al.*[6,7]. The mechanism of action,[8~10] toxicity[11,12] and pharmacokinetics[13,14] of tallysomycin A have been studied. The ^{13}C NMR assignments of tallysomycin A were recently described by GREENAWAY *et al.*[15].

It has been reported[16] that, in the bleomycin fermentation, certain amines added to the fermentation medium were incorporated into the terminal amine moiety of bleomycin molecule, affording new bleomycin derivatives. Addition of a variety of diamines to the tallysomycin fermentation medium resulted in the formation of two series of new tallysomycin derivatives, both containing the precursor amine

Fig. 1. Structures of tallysomycins A and B.



* The USAN generic name "Talisomycin" has been assigned to tallysomycin A.

in the terminal position but differing in the presence or absence of a β-lysine moiety in the subterminal position. The new biosynthetic tallysomycin derivatives are designated as tallysomycin S, followed by a suffix number to distinguish the terminal amine moiety and a suffix letter of "a" or "b" to show whether it is the analog of tallysomycin A or B (*i.e.* the presence or absence of β-lysine moiety).

This paper reports the production, isolation and characterization of a series of biosynthetic tallysomycin derivatives. The antimicrobial and antitumor activities of the new derivatives are also described.

Materials and Methods

Precursor Amines

The precursor amine compounds used in the present study are listed in Table 1. Most of the amines tested were commercially available except for N-(α-phenylethyl)-1,3-diaminopropane and 3-aminopropyldimethylsulfonium chloride, which were prepared in our laboratories according to published method.[17,18]

Fermentation

An agar slant of *Streptoalloteichus hindustanus* strain E465-94 (ATCC 31158) was used to inoculate vegetative medium containing 1.5% glucose, 0.2% yeast extract, 0.5% peptone, 0.05% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.5% $CaCO_3$, the pH being adjusted to 7.2 before sterilization. The seed culture was incubated at 28°C for 48 hours on a rotary shaker (250 rpm), and 10 ml of the growth was transferred to a 500-ml Erlenmeyer flask containing 100 ml of the fermentation medium composed of 2.5% sucrose, 0.5% glucose, 3% cottonseed meal, 3% distiller's solubles, 0.3% $(NH_4)_2SO_4$, 0.003% $ZnSO_4 \cdot 7H_2O$, 0.01% $CuSO_4 \cdot 5H_2O$ and 0.4% $CaCO_3$. A precursor amine compound was added to the medium in the form of a neutralized aqueous solution at concentrations of 0.1~0.2% (w/v). The pH of the fermentation medium was adjusted to pH 6.5 before sterilization. Antibiotic production in shake cultures generally reached a maximum after 5~6 days at 28°C.

Assay of Antibacterial Activity

Antibiotic levels in fermentation broths and the extracts of tallysomycin derivatives were determined by a paper disc-agar diffusion method using *Mycobacterium smegmatis* strain M6-3 as the test organism. This is a laboratory-developed aminoglycoside-resistant mutant derived from *M. smegmatis* ATCC 607. It is insensitive to nebramycin factors which are generally co-produced[1] with tallysomycin. Tallysomycin A hydrochloride was used as the assay standard, the potency of copper-free tallysomycin A free base being defined as 1,000 units/mg. Test and standard samples were diluted with M/15 phosphate buffer of pH 7.0.

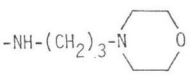
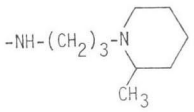
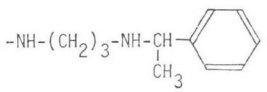
Isolation and Purification

Harvested broth was filtered with filter aid and the bioactivity in the filtrate adsorbed on a column of Amberlite IRC-50 (60% NH_4^+ form) at pH 7.0. The column was washed with water and then developed with 0.1 N hydrochloric acid. The active fractions were collected, neutralized with aqueous ammonia and adsorbed on a column of activated charcoal. The charcoal was washed with water and tallysomycin eluted with a 1:1 mixture of *n*-butanol and diluted HCl solution (pH 2.0). The butanol layer was separated, and the aqueous layer was neutralized with Amberlite IR-45 (OH^- form) and concentrated *in vacuo*. The aqueous concentrate was added to 5% $CuCl_2$ solution and applied on a column of Diaion HP-20. The column was developed with water, and the active eluate was concentrated *in vacuo* and lyophilized to give a blue powder of tallysomycin complex in a copper-chelated form. The antibiotic complex thus obtained was separated by CM-Sephadex C-25 column chromatography using increasing concentrations of aqueous ammonium formate solution (1~7%). The fractions were monitored by bioassay as well as by optical density at 290 nm. Each component was further purified by desalting on a column of Diaion HP-20.

Identification of New Tallysomycin Derivatives

The purity and identity of tallysomycin derivatives was examined by thin-layer chromatography (TLC) using a silica gel plate (Kiesel gel $60F_{254}$, Merck) and a solvent system of MeOH - 10% $AcONH_4$ =

Table 1. Precursor amines and biosynthetic tallysomycin derivatives.

Precursor amines	Name of new tallysomycins produced	Terminal amine structure
1,3-Diaminopropane	S_{1a}, S_{1b}	$-NH-(CH_2)_3-NH_2$
3-Aminopropyl-dimethylsulfonium chloride	S_{2a}, S_{2b}	$-NH-(CH_2)_3-S^+(CH_3)_2$
Ethylenediamine	S_{3b}	$-NH-(CH_2)_2-NH_2$
1,3-Diamino-2-hydroxypropane	S_{4b}	$-NH-CH_2-CH(OH)-CH_2-NH_2$
N-(β-Hydroxypropyl)-1,2-diaminoethane	S_{5b}	$-NH-(CH_2)_2-NH-CH_2-CH(OH)-CH_3$
N-(β-Hydroxyethyl)-1,3-diaminopropane	S_{6a}, S_{6b}	$-NH-(CH_2)_3-NH-CH_2-CH_2OH$
N,N-Dimethyl-1,3-diaminopropane	S_{7b}	$-NH-(CH_2)_3-N(CH_3)_2$
N,N-Di(β-hydroxyethyl)-1,3-diaminopropane	S_{8a}, S_{8b}	$-NH-(CH_2)_3-N(CH_2-CH_2OH)_2$
N-(β-Hydroxyethyl)-1,2-diaminoethane	S_{9b}	$-NH-(CH_2)_2-NH-CH_2-CH_2OH$
1,4-Diaminobutane	S_{10a}, S_{10b}	$-NH-(CH_2)_4-NH_2$
N-Methyl-1,3-diaminopropane	S_{11a}, S_{11b}	$-NH-(CH_2)_3-NH-CH_3$
N-(3-Aminopropyl)morpholine	S_{12b}	
N-(3-Aminopropyl)-2-pipecoline	S_{13b}	
N-(α-Phenylethyl)-1,3-diaminopropane	S_{14a}, S_{14b}	

1:1 (S-102) or MeOH - 10% $AcONH_4$ - 10% NH_4OH=10:9:1 (S-123). Detection was made by a dual-wavelength TLC scanner (Shimadzu CS-910) at 290 nm.

High-performance liquid chromatography (HPLC) was also employed to identify new biosynthetic tallysomycins. The HPLC apparatus used was a Model ALC204 of Waters Associates equipped with a Type U6K injector and a Model 440 UV-detector operating at 254 nm. A column of μ Bondapak C_{18} (Waters, 300×4 mm I.D.) was used. The mobile phase was a 30% aqueous acetonitrile solution containing PIC reagent B-7 (Waters). The flow rate was 1.0 ml/minute under a pressure of 800 p.s.i. The sample size injected was 1 μl of 2 mg/ml solution.

For the identification of the terminal amine moiety of tallysomycins, each component was hydrolyzed with 6 N HCl at 105°C for 17 hours and the amine or amino acid liberated in the hydrolyzate was examined by TLC using systems S-102 and S-123. The presence or absence of β-lysine in the hydrolyzate was also determined simultaneously.

Determination of Antimicrobial Activity

The minimum inhibitory concentrations (MIC) of tallysomycin derivatives were determined against Gram-positive, Gram-negative, acid-fast bacteria and fungi by a two-fold agar dilution method using a Steer's multiple inoculator. Nutrient agar was used for bacteria and SABOURAUD agar for fungi.

The ability to induce prophage in a lysogenic bacterium, *E. coli* W1709 (λ), was determined according to the method of LEIN *et al.*[19]. Plaque counts were made on agar plates containing the test compound (T) and on control plates (C). A T/C ratio of the plaque counts of greater than 3.0 was considered significant and the lysogenic induction activity (ILB activity) was expressed as the minimum inducible concentration of the test compound.

Table 2. Physico-chemical properties of tallysomycin derivatives.

Tallysomycin	TLC (Rf)		HPLC (Retention time)	UV spectrum
	S-102	S-123		$\lambda_{max}^{H_2O}$ nm, ($E_{1cm}^{1\%}$)
A	0.22	0.05	8′30″	243 (125), 291 (98)
B	0.41	0.11	6′30″	243 (143), 291 (109)
S_{1a}	0.50	0.23	6′24″	242 (126), 292 (102)
S_{1b}	0.65	0.37	4′48″	242 (108), 290 (94)
S_{2a}	0.24	0.15	6′30″	240 (120), 290 (108)
S_{2b}	0.38	0.34	5′18″	244.5 (129), 292 (104)
S_{3b}	0.59	0.51	4′49″	240 (123), 291 (112)
S_{4b}	0.66	0.40	4′47″	242 (120), 290 (103)
S_{5b}	0.50	0.58	4′54″	244 (124), 290 (102)
S_{6a}	0.42	0.22	6′18″	243 (105), 291 (83)
S_{6b}	0.50	0.40	4′42″	243 (128), 291 (103)
S_{7b}	0.44	0.25	4′54″	245 (120), 290 (96)
S_{8a}	0.56	0.40	6′24″	243 (107), 291 (85)
S_{8b}	0.70	0.54	4′48″	243 (114), 290 (92)
S_{9b}	0.56	0.48	4′52″	243 (104), 290 (87)
S_{10a}	0.43	0.24	6′20″	244 (142), 292 (112)
S_{10b}	0.61	0.39	4′47″	244 (141), 292 (116)
S_{11a}	0.27	0.16	—	244 (62), 292 (53)
S_{11b}	0.49	0.35	4′47″	244 (112), 292 (91)
S_{12b}	0.49	0.60	4′47″	245 (74), 292 (59)
S_{13b}	0.46	0.36	5′37″	243 (142), 292 (114)
S_{14a}	0.52	0.37	—	243 (102), 292 (72)
S_{14b}	0.62	0.50	7′05″	243 (143), 292 (109)

Determination of Antitumor Activity

The antitumor activity of tallysomycin derivatives was examined in three experimental tumor systems in mice. Lymphocytic leukemia P388 was implanted into the peritoneum of BDF_1 mice of either sex at an inoculum size of 3×10^5 cells per mouse. Sarcoma 180 ascites tumor was inoculated intraperitoneally into male *dd*-strain mice with 2.5×10^6 cells per mouse. Melanotic melanoma B16 was implanted subcutaneously into BDF_1 mice using 5×10^5 cells per mouse.

Twenty-four hours after the implantation of tumor cells, graded doses of test compounds were administered to mice intraperitoneally in an injection volume of 0.2 ml per 10 g of body weight. Test compounds were given once daily for 9 days (*qd* 1→9 schedule). Death or survival of the treated and non-treated (control) animals was recorded daily during the observation period of 45 days after the implantation of tumor cells, and the median survival time was calculated for each of the test (T) and control (C) groups. A T/C value equal to or greater than 125% against leukemia P388 was considered to be a significant antitumor effect. The actual dose giving a T/C of 125% was estimated by linear regression analysis and defined as the effective dose 125 or ED_{125}. The effective dose 150 (ED_{150}) was employed for the evaluation of antitumor effect against sarcoma 180. In the B16 melanoma experiment the tumor size was measured on day 16 after tumor inoculation, and the dose giving 50% inhibition of tumor growth (ID_{50}) was calculated from regression lines.

Acute Toxicity Determination

Graded doses of tallysomycin derivatives were administered intraperitoneally to groups of *dd* mice. Death or survival of the animals was recorded for 30 days and the LD_{50} (median lethal dose) was calculated according to the method of VAN DER WAERDEN.[20]

Table 3. Antimicrobial and ILB activities to tallysomycin derivatives.

Tallysomycin	Antimicrobial activity* (MIC in mcg/ml)				ILB activity** (mcg/ml)
	S. aureus Smith	*E. coli* NIHJ	*M. smegmatis* ATCC 607	*A. fumigatus* IAM 2530	
A	0.1	0.05	0.2	1.6	0.00125
B	0.4	0.2	0.2	0.8	0.01
S_{1a}	0.4	0.1	0.012	0.8	0.16
S_{1b}	1.6	0.4	<0.05	1.6	0.31
S_{2a}	0.2	<0.05	<0.05	0.4	0.31
S_{2b}	1.6	0.4	0.2	0.8	0.63
S_{3b}	0.8	0.1	0.05	0.8	0.31
S_{4b}	0.8	0.4	0.003	0.8	—
S_{5b}	6.3	0.4	0.2	0.4	0.08
S_{6a}	0.8	0.4	0.4	0.2	0.02
S_{6b}	6.3	0.4	0.2	0.8	0.08
S_{7b}	6.3	0.4	3.1	0.4	0.08
S_{8a}	0.2	0.1	0.1	0.8	0.005
S_{8b}	0.4	0.2	0.1	1.6	0.02
S_{9b}	0.4	0.1	0.1	1.6	0.16
S_{10a}	1.6	0.4	0.2	1.6	0.16
S_{10b}	6.3	0.8	0.2	6.3	0.31
S_{11a}	0.8	0.1	0.1	0.8	0.08
S_{11b}	3.1	0.2	0.1	6.3	0.63
S_{12b}	12.5	1.6	0.2	25	0.31
S_{13b}	6.3	0.4	0.2	12.5	0.08
S_{14a}	0.8	0.2	0.1	3.1	0.01
S_{14b}	3.1	0.4	0.2	25	0.04

* Determined in nutrient agar.
** Minimum inducible concentration.

Results and Discussion

A number of new tallysomycin derivatives were produced when certain diamines were added to the fermentation medium. Under the fermentation conditions used in the present study, tallysomycin B derivatives (S_b series) were produced more predominantly than those of tallysomycin A (S_a series). In some cases only the former type of analog was obtained. Tallysomycins A and B were coproduced as minor components in the precursor-fed fermentation. Table 1 shows the biosynthetic tallysomycin derivatives isolated along with the structure of terminal amine moiety. The physico-chemical properties of tallysomycin derivatives are listed in Table 2.

The antimicrobial activities of tallysomycin derivatives determined by means of a serial agar dilution method are shown in Table 3. They were active against Gram-positive, Gram-negative and acid-fast bacteria. A fungal strain, *Aspergillus fumigatus*, was inhibited at low concentrations of all derivatives except for tallysomycins S_{12b}, S_{13b} and S_{14b}. The new tallysomycin derivatives showed the ability to induce prophage of lysogenic bacteria as is shown in Table 3.

The antitumor activities of tallysomycin derivatives determined in three tumor systems are shown in Table 4. The acute toxicities of tallysomycin derivatives for mice after a single intraperitoneal admini-

Table 4. Antitumor activity and acute toxicity of tallysomycin derivatives.

Tallysomycin	Antitumor activity (mg/kg/day, i.p.)			Toxicity LD_{50} (mg/kg, i.p.)
	P388 (ED_{125})	S180 (ED_{150})	B16 (ID_{50})	
A*	0.26	0.07	0.27	19
B*	0.64	0.06	0.72	46
S_{1a}	1.2	0.07	0.84	25
S_{1b}	2.0	0.11	0.15	19
S_{2a}	3.0	0.07	0.17	25
S_{2b}	0.91	0.12	0.33	32
S_{3b}	0.32	0.06	0.20	19
S_{4b}	3.0	0.13	0.50	13
S_{5b}	1.7	0.10	0.60	46
S_{6a}	0.70	0.09	0.12	15
S_{6b}	2.2	0.08	0.22	30
S_{7b}	2.1	0.14	0.16	30
S_{8a}	0.78	—	—	42
S_{8b}	1.5	0.25	0.26	30
S_{9b}	3.0	0.42	0.29	32
S_{10a}	0.28	0.08	0.39	27
S_{10b}*	0.47	0.02	0.47	42
S_{11a}	0.60	—	—	18
S_{11b}	0.28	0.20	0.70	21
S_{12b}	1.30	0.06	1.30	>50
S_{13b}	0.70	0.33	0.23	35
S_{14a}	—	—	—	—
S_{14b}	3.0	—	0.43	—

* Copper-free form; others are copper-complex.

Table 5. Antitumor therapeutic indices of tallysomycin derivatives.

Tallysomycin	Antitumor therapeutic indices*		
	P388	S180	B16
A	73	271	70
B	72	767	64
S_{1a}	21	357	30
S_{1b}	10	173	127
S_{2a}	8	357	147
S_{2b}	35	267	97
S_{3b}	59	317	95
S_{4b}	4	100	26
S_{5b}	27	460	77
S_{6a}	21	167	125
S_{6b}	14	375	136
S_{7b}	14	214	188
S_{8a}	54	—	—
S_{8b}	20	120	115
S_{9b}	11	76	110
S_{10a}	96	338	69
S_{10b}	89	2,100	89
S_{11a}	30	—	—
S_{11b}	75	105	30
S_{12b}	>38	>833	>38
S_{13b}	50	106	152
S_{14a}	—	—	—
S_{14b}	—	—	—

* $\dfrac{\text{single dose toxicity } (LD_{50})}{\text{antitumor effective dose } (ED_{125}, ED_{150} \text{ or } ED_{50})}$

stration are also shown in Table 4. The antitumor therapeutic index was calculated for each derivative from the ratio of toxicity and antitumor activity. As shown in Table 5, several of the new biosynthetic tallysomycin derivatives demonstrated better therapeutic indices than the naturally obtained tallysomycin A or B in some of the experimental tumor systems examined. Among these, tallysomycin S_{10b} having 1,4-diaminobutane as the terminal amine was selected for further study in view of its favorable therapeutic index.

Acknowledgement

The authors wish to thank Messrs. T. TSUNO, I. YAMAURA and S. ANDO for their contributions to the present study.

References

1) KAWAGUCHI, H.; H. TSUKIURA, K. TOMITA, M. KONISHI, K. SAITO, S. KOBARU, K. NUMATA, K. FUJISAWA, T. MIYAKI, M. HATORI & H. KOSHIYAMA: Tallysomycin, a new antitumor antibiotic complex related to bleomycin. I. Production, isolation and properties. J. Antibiotics 30: 779~788, 1977

2) TOMITA, K.; Y. UENOYAMA, K. NUMATA, T. SASAHIRA, Y. HOSHINO, K. FUJISAWA, H. TSUKIURA & H. KAWAGUCHI: *Streptoalloteichus*, a new genus of the family *Actinoplanaceae*. J. Antibiotics 31: 497~510, 1978
3) KONISHI, M.; K. SAITO, K. NUMATA, T. TSUNO, K. ASAMA, H. TSUKIURA, T. NAITO & H. KAWAGUCHI: Tallysomycin, a new antitumor antibiotic complex related to bleomycin. II. Structure determination of tallysomycins A and B. J. Antibiotics 30: 789~805, 1977
4) TAKITA, T.; Y. MURAOKA, T. NAKATANI, A. FUJII, Y. UMEZAWA, H. NAGANAWA & H. UMEZAWA: Chemistry of bleomycin. XIX. Revised structures of bleomycin and phleomycin. J. Antibiotics 31: 801~804, 1978
5) IMANISHI, H.; M. OHBAYASHI, Y. NISHIYAMA & H. KAWAGUCHI: Tallysomycin, a new antitumor antibiotic complex related to bleomycin. III. Antitumor activity of tallysomycins A and B. J. Antibiotics 31: 667~674, 1978
6) BRADNER, W. T.; H. IMANISHI, R. S. HIRTH & I. WODINSKY: Antitumor activity of Bu-2231 A, a new bleomycin analogue. Proc. Am. Assoc. Cancer Res. 18: 35, 1977
7) BRADNER, W. T.: Bu-2231, a third-generation bleomycin: Preclinical studies. *In* The Bleomycin—Current Status and New Developments. *Eds.*, CARTER, S.; S. T. CROOKE & H. UMEZAWA, pp. 333~342. Academic Press Inc., New York, NY, 1978
8) STRONG, J. E. & S. T. CROOKE: DNA breakage by tallysomycin. Cancer Res. 38: 3322~3326, 1978
9) STRONG, J. E. & S. T. CROOKE: Mechanism of action of tallysomycin, a third generation bleomycin. *In* The Bleomycin—Current Status and New Developments. *Eds.*, CARTER S.; S. T. CROOKE, & H. UMEZAWA, pp. 343~355, Academic Press Inc., New York, NY, 1978
10) LOWN, J. W. & A. V. JOSHUA: Interactions of the glycopeptide antitumor antibiotics bleomycin and tallysomycin with deoxyribonucleic acid *in vitro*. Biochem. Pharmacol. 29: 521~532, 1980
11) SIKIC, B. I.; Z. H. SIDDIK & T. E. GRAM: Relative pulmonary toxicity and antitumor effects of two new bleomycin analogs, pepleomycin and tallysomycin A. Cancer Treat. Rep. 64: 659~667, 1980
12) SCHLEIN, A.; J. E. SCHURIG, C. D. BACA, W. T. BRADNER & S. T. CROOKE: Pulmonary toxicity studies of bleomycin and tallysomycin A in mice. to be published.
13) STRONG, J. E.; J. E. SCHURIG, B. F. ISSELL, W. G. KRAMER, A. F. TAVEL, A. P. FLORCZYK & S. T. CROOKE: Pharmacokinetics of tallysomycin and bleomycin in the beagle dog. Cancer Treat. Rep. 63: 1821~1827, 1979
14) VAN HARKEN, D. R.; R. D. SMYTH, F. H. LEE, E. F. CHRISTENSEN, J. E. STRONG, S. T. CROOKE & G. H. HOTTENDORF: Pharmacokinetics of tallysomycin in the rhesus monkey. Cancer Treat. Rep. to be published
15) GREENAWAY, F. T.; J. C. DABROWIAK, R. GRULICH & S. T. CROOKE: The ^{13}C NMR spectra of tallysomycin and its zinc (II) complex. Org. Mag. Reson. 13: 270~273, 1980
16) FUJII, A.; T. TAKITA, N. SHIMADA & H. UMEZAWA: Biosynthesis of new bleomycins. J. Antibiotics 27: 73~77, 1974
17) SUYAMA, T. & S. KANAO: Decarboxylation of amino acids. II. 3-Methylthiopropylamine. Yakugaku Zasshi 84: 1012~1014, 1964
18) HLAVKA, J. J.; P. BITHA, J. BOOTHE & T. FIELDS: Glycocinnamoylspermidines, a new class of antibiotics. IV. Chemical modification of LL-BM 123γ. J. Antibiotics 31: 477~479, 1978
19) LEIN, J.; B. HEINEMANN & A. GOUREVITCH: Induction of lysogenic bacteria as a method of detecting potential antitumor agents. Nature 196: 783~784, 1962
20) VAN DER WAERDEN, B. L.: Wirksamkeits- und Konsentrationsbestimmung durch Thierversuche. Arch. Exp. Path. Pharmak. 195: 389~412, 1940