LEPTOLSTATIN FROM *Streptomyces* sp. SAM1595, A NEW GAP PHASE-SPECIFIC INHIBITOR OF THE MAMMALIAN CELL CYCLE

II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE

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(Received for publication November 16, 1992)

Leptolstatin, a product from *Streptomyces* sp. SAM1595, is a gap phase-specific inhibitor of the mammalian cell cycle. Physico-chemical properties and spectrometric analyses showed that the structure of leptolstatin is (2*Z*,6*E*,8*Z*,12*E*,14*E*,22*E*)-19,24-dihydroxy-8,10,14,16,18,20,22-heptamethyl-17-oxo-2,6,8,12,14,22-tetracosahexaen-5-olide.

Several secondary metabolites from actinomycetes have recently been found to inhibit proliferation of cultured animal cells by blocking the cell cycle progression in G1 and G2 phases[1~4]. Leptomycin B is one of such compounds possessing a unique structure of a long branched dicarboxylic acid, in which one carboxyl group formed a δ-lactone ring[3,5,6]. In the course of our screening program for the gap phase-specific inhibitors of the mammalian cell cycle, we found that *Streptomyces* sp. SAM1595 produced an active substance whose structure was similar to leptomycins but contained a primary alcoholic group instead of one of the two carboxyl groups. The compound was named leptolstatin according to the structural similarity to leptomycins. Taxonomy of the producing strain, fermentation, purification, and biological activities have been reported in the preceding paper[1]. This paper deals with the physico-chemical properties and structural elucidation of leptolstatin.

Materials and Methods

The UV and IR spectra were recorded on a Shimadzu UV-240 and a NICOLET 7199 (FT)-IR spectrometer, respectively. The ^{1}H NMR (500 MHz) and ^{13}C NMR (67.8 MHz) spectra were obtained with a General Electric GN-500 FT NMR spectrometer and a JEOL EX-270 FT NMR spectrometer, respectively. The fast atom bombardment mass spectra (FAB-MS) were measured with a JEOL HX 110 spectrometer using 2,2-dithiodiethanol as the matrix.

Results and Discussion

Physico-chemical properties of leptolstatin are summarized in Table 1. The molecular formula of leptolstatin was determined to be $C_{31}H_{46}O_5$ by the analysis of high-resolution FAB-MS, ^{1}H NMR and

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Table 1. Physico-chemical properties of leptolstatin.

Appearance	Colorless oil
$[\alpha]_D$ (MeOH)	$-130°$ (c 0.18)
Molecular formula	$C_{31}H_{46}O_5$
HRFAB-MS	Obsd: 505.3507 $(M+Li)^+$
	Calcd. for $C_{31}H_{46}O_5Li$: 505.3509
Solubility Soluble	$CHCl_3$, EtOAc, acetone, MeOH
Insoluble	Hexane, H_2O
UV λ_{max}^{MeOH} nm	243 (22,000), 296 (sh, 1,000)
IR ν_{max}^{film} cm^{-1}	3430, 2960, 2920, 2850, 1730, 1710, 1460, 1380, 1250, 1100, 1060, 970

Table 2. ^1H NMR assignments for leptolstatin and leptomycin B ($CDCl_3$).

Position	Chemical shifts (ppm)	
	Leptolstatin	Leptomycin B
2	6.06 d	5.98 d
3	6.90 m	6.94 dd
4	2.47 m 2H	2.53 m
5	4.98 dd	4.99 dd
6	5.72 dd	5.71 dd
7	6.73 d	6.63 d
9	5.27 d	5.22 d
10	2.67 m	2.67 m
11	2.08 m 2H	2.09 t 2H
12	5.59 m	5.59 m
13	6.00 d	5.98 d
15	5.10 d	5.07 d
16	3.66 m	3.66 m
18	2.85 m	2.82 m
19	3.59 t	3.57 t
20	1.60 m	1.75 m
21a	1.75 m	1.90 dd
21b	2.04 m	2.21 dd
23	5.41 t	5.66 s
24	4.16 m	
4-CH_3		1.06 d 3H
8-CH_3	1.83 d 3H	
8-CH_2CH_3		2.20 q 2H
8-CH_2CH_3		1.04 t 3H
10-CH_3	0.97 d 3H	0.96 d 3H
14-CH_3	1.83 d 3H	1.81 d 3H
16-CH_3	1.13 d 3H	1.13 d 3H
18-CH_3	1.15 d 3H	1.14 d 3H
20-CH_3	0.79 d 3H	0.77 d 3H
22-CH_3	1.63 s 3H	2.10 s 3H

Table 3. ^{13}C NMR data of leptolstatin and leptomycin B ($CDCl_3$).

Carbon	Function group	Carbon shift value (ppm)	
		Leptolstatin	Leptomycin B
C-1	CO	174.3 s	164.4 s
C-2	CH	121.6 d	120.0 d
C-3	CH	144.8 d	151.6 d
C-4	CH_2	30.1 t	
C-4	CH		33.6 d
C-5	CH	78.7 d	81.5 d
C-6	CH	125.3 d	122.8 d
C-7	CH	130.9 d	130.2 d
C-8	C	129.5 s	135.6 s
C-9	CH	139.0 d	136.9 d
C-10	CH	32.3 d	32.2 d
C-11	CH_2	40.7 t	40.9 t
C-12	CH	127.8 d	128.2 d
C-13	CH	135.3 d	135.3 d
C-14	C	137.7 s	136.5 s
C-15	CH	128.3 d	128.0 d
C-16	CH	45.7 d	45.7 d
C-17	CO	215.5 s	214.9 s
C-18	CH	46.8 d	47.0 d
C-19	CH	73.8 d	74.2 d
C-20	CH	33.1 d	33.6 d
C-21	CH_2	44.0 t	45.7 t
C-22	C	136.3 s	160.9 s
C-23	CH	125.6 d	117.1 d
C-24	CH_2	59.2 t	
C-24	C		171.3 s
C-4Me	CH_3		12.3 q
C-8Me	CH_3	20.3 q	
C-8Et	CH_2		26.6 t
	CH_3		13.5 q
C-10Me	CH_3	20.7 q	13.0 q
C-14Me	CH_3	13.1 q	18.5 q
C-16Me	CH_3	16.1 q	13.0 q
C-18Me	CH_3	12.7 q	20.9 q
C-20Me	CH_3	13.8 q	13.6 q
C-22Me	CH_3	15.8 q	16.0 q

^{13}C NMR spectra (Tables 1~3).

Since the UV (Table 1), ^1H NMR and IR spectra (Figs. 1 and 2) of leptolstatin resembled those of leptomycin B[5], leptolstatin is supposed to possess a structure similar to those of leptomycins.

To determine the structural difference between leptomycin B and leptolstatin, we compared ^1H NMR and ^{13}C NMR spectral data of these compounds (Tables 2 and 3). In the ^{13}C and ^1H NMR spectra of leptolstatin, a new methylene signal was observed at δ_C 30.1 and δ_H 2.47 in place of a methine (δ_C 33.6, δ_H 2.53) and methyl signals at C-4 (δ_C 12.3, δ_H 1.06) found in the spectrum of leptomycin B, indicating that the C-4 position of leptolstatin is demethylated. In addition, a vinylic methyl group (δ_C 20.3, δ_H 1.83) was newly observed in leptolstatin instead of the

Fig. 1. ^1H NMR spectrum of leptolstatin ($CDCl_3$, 500 MHz).

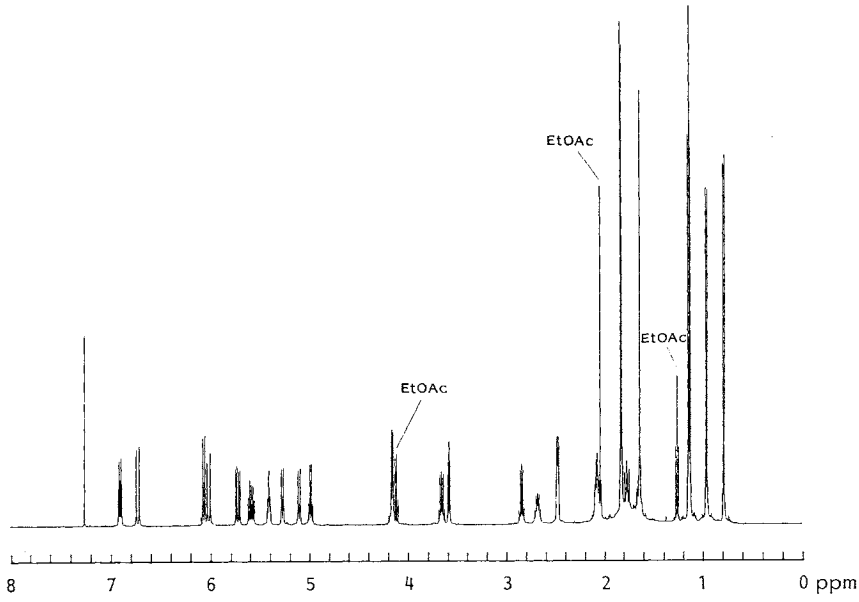
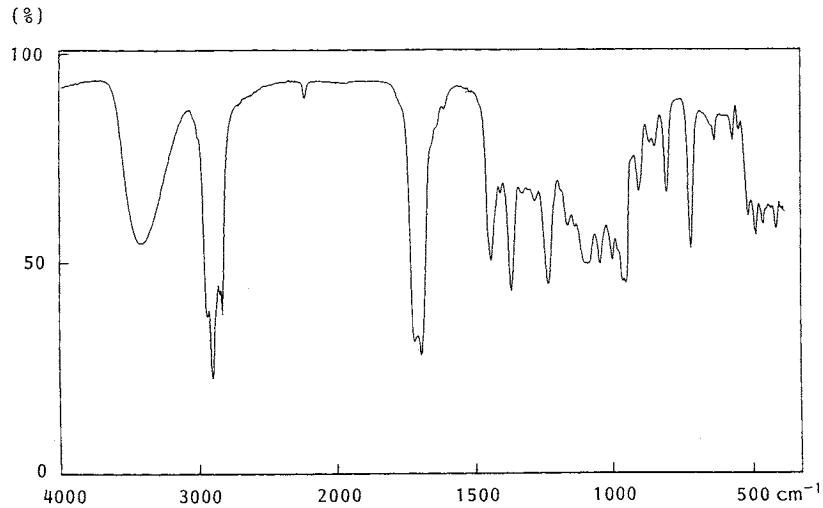


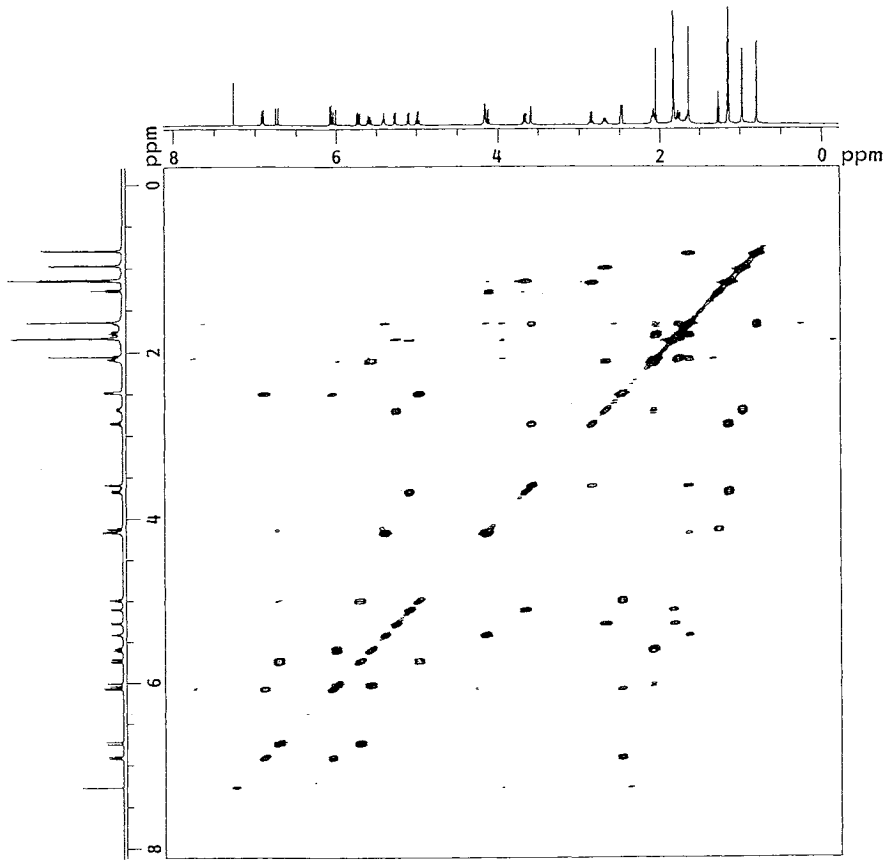
Fig. 2. IR absorption spectrum of leptolstatin (film).



C-8 ethyl group of leptomycin B. Furthermore, a new methylene signal (δ_C 59.2, δ_H 4.16) was detected in place of the C-24 tertiary carbon signal, suggesting that leptolstatin contains a hydroxymethyl group instead of the terminal carboxyl group of leptomycin B. The IR spectrum of leptolstatin supported the absence of the carboxyl group (Fig. 2). These data indicate that leptolstatin is a new leptomycin B analogue, in which the C-4 position is demethylated and the C-8 ethyl and the terminal carboxyl groups are replaced by the methyl and the hydroxymethyl groups, respectively. This structure was further confirmed by 2D correlation spectroscopy (COSY) (Fig. 3).

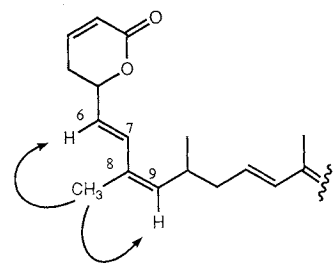
In the previous studies on leptomycins and other related compounds, geometry of a double bond

Fig. 3. ^1H-^1H COSY spectrum of leptolstatin ($CDCl_3$, 500 MHz).



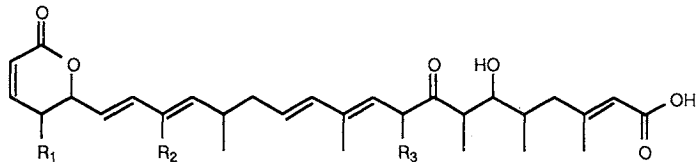
between C-8 and C-9 had not yet been determined. We analyzed the configuration in the structure of leptolstatin by nuclear Overhauser enhancement (NOE). As the NOE signals were observed between 9-H and 8-CH_3 as well as 6-H and 8-CH_3, the double bond between C-8 and C-9 was assigned as a *Z* form (Fig. 4). Thus we concluded that the structure of leptolstatin was (2*Z*,6*E*,8*Z*,12*E*,14*E*,22*E*)-19,24-dihydroxy-8,10,14,16,18,20,22-heptamethyl-17-oxo-2,6,8,12,14,22-tetracosahexaen-5-olide, as shown in Fig. 5.

Fig. 4. NOE found in ^1H NMR spectrum of leptolstatin.

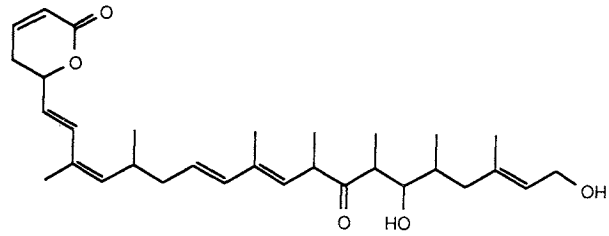


Six antibiotics structurally related to leptolstatin have so far been reported, namely leptomycins A and B[5,6] (PD 114,720[7], CI-940[7], elactocin[7], CL-1957A[8], jildamycin[9], matuamycin[9]), kazusamycins A and B[10] (PD 114,721[7], hydroxyelactocin[7], CL-1957E[11]), and anguinomycins A and B[12], all of which possess a terminal carboxyl group (Fig. 5). Leptolstatin is a novel member of this class of related compounds with a unique structure in which the terminal carboxyl group is replaced by a hydroxymethyl group. Previous studies by means of chemical modification of kazusamycins showed that the terminal carboxyl group was necessary for the activity because methyl esterified kazusamycin at the terminal carboxyl group

Fig. 5. Structure of leptolstatin and its related compounds.



	R_1	R_2	R_3
Leptomycin A	CH_3	CH_3	CH_3
Leptomycin B	CH_3	CH_2CH_3	CH_3
Kazusamycin A	CH_3	CH_2CH_3	CH_2OH
Kazusamycin B	CH_3	CH_3	CH_2OH
Anguinomycin A	H	CH_3	CH_3
Anguinomycin B	H	CH_2CH_3	CH_3



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possessed only weak activity[13]. However, the present study suggests that the terminal carboxyl group is non-essential for the expression of the biological activity. An alternative possibility is the oxidation of the hydroxymethyl group of leptolstatin after incorporation into the cells, thereby leptolstatin is converted to an active form possessing a terminal carboxyl group.

The NOE study showed that a double bond between C-8 and C-9 of leptolstatin was a *Z* form, which had not been assessed in the case of leptomycins or other compounds. A similar NOE analysis was performed on leptomycin B, and the corresponding double bond was also found to be *Z* (data not shown). It therefore seems highly probable that all the members belonging to this group possess the identical skeleton with a *Z* form at C-8/C-9, which can be determined by the NOE analysis.

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