Preparation of Leustroducsin H and the Structure-activity Relationship of Its Derivatives

TOMOYUKI SHIBATA*, SHINWA KURIHARA, TETSUO OIKAWA, NOBUYUKI OHKAWA, NAOMI SHIMAZAKI†, KAZUHIKO SASAGAWA†, TOMOWO KOBAYASHI†, TAKAFUMI KOHAMA††, FUMITOSHI ASAI††, AKIO SHIRAISHI† and YUKIO SUGIMURA

Exploratory Chemistry Research Laboratories, Biological Research Laboratories† and Pharmacology and Molecular Biology Research Laboratories††, Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan

(Received for publication July 14, 1995)

Leustroducsins (LSN) A, B and C[1,2] (**1a**, **1b** and **1c**, respectively) has been identified as new potent cytokine inducers produced by *Streptomyces platensis* SANK 60191. The compounds were shown to increase the levels of several cytokines including granulocyte (G) colony-stimulating factor (CSF), granulocyte-macrophage (GM)-CSF, and interleukins (IL)-6, and IL-11 in bone marrow stromal cells. Amoung these cytokines, IL-6[3] and IL-11[4] are reported to increase platelet levels *in vivo*. In our previous paper[5] we have reported that LSN-B exhibited platelet-elevating activity in mice. But, since LSNs are produced in certain microorganisms as a complex mixture, the task of obtaining each LSN separately in large scale was found to be arduous, and development of an efficient method of generating a single congener of LSN in large scale is required. Herein we disclose the preparation of LSN-H (**1d**), a derivative enzymatically prepared by removing acyl moieties from the cyclohexane ring of LSNs, and its thrombopoietic effects. Furthermore we also report on the structure-activity relationship of its derivatives.

A mixture of LSNs was treated with porcine liver esterase (PLE-A) in acetone-phosphate buffer (pH = 6.7) at 37°C, after filtering off the enzyme, purification by Cosmosil column afforded LSN-H (**1d**) along with small amount of alcohol **2**. The hydrolysis to yield **1d** only indicated that all LSNs have the same absolute configuration. The absolute configuration of LSN-H was determined to be 4*S*, 5*S*, 8*R*, 9*R*, 11*R*, 16*R*, 18*S*.[6]

N-acetyl LSN-H (**3a**) was synthesized by treating LSN-H with N-methoxydiacetamide in methanol. Compounds **3b**~**3l** were prepared by treating LSN-H with the corresponding *p*-nitrophenyl esters in methanol. Elementary analysis of compounds **3a**~**3l** indicated that these compounds pertain to be in their sodium salt form, presumably formed during Cosmosil column chromatography. Compounds **3m**~**3n** were synthesized by treating LSN-H with the corresponding acid chlorides in the presence of sodium bicarbonate in dioxane-water. Compound **3o** was obtained by treating LSN-H with di-t-butyl dicarbonate in the presence of sodium bicarbonate in dioxane-water. Treatment of **3o** with acid chloride in the presence of triethylamine in dichloromethane gave cyclic phosphate **3p**. Dephosphorylation of **1d** in refluxing formamide-phosphate

Fig. 1. Structure of the leustroducsins and their related compounds.

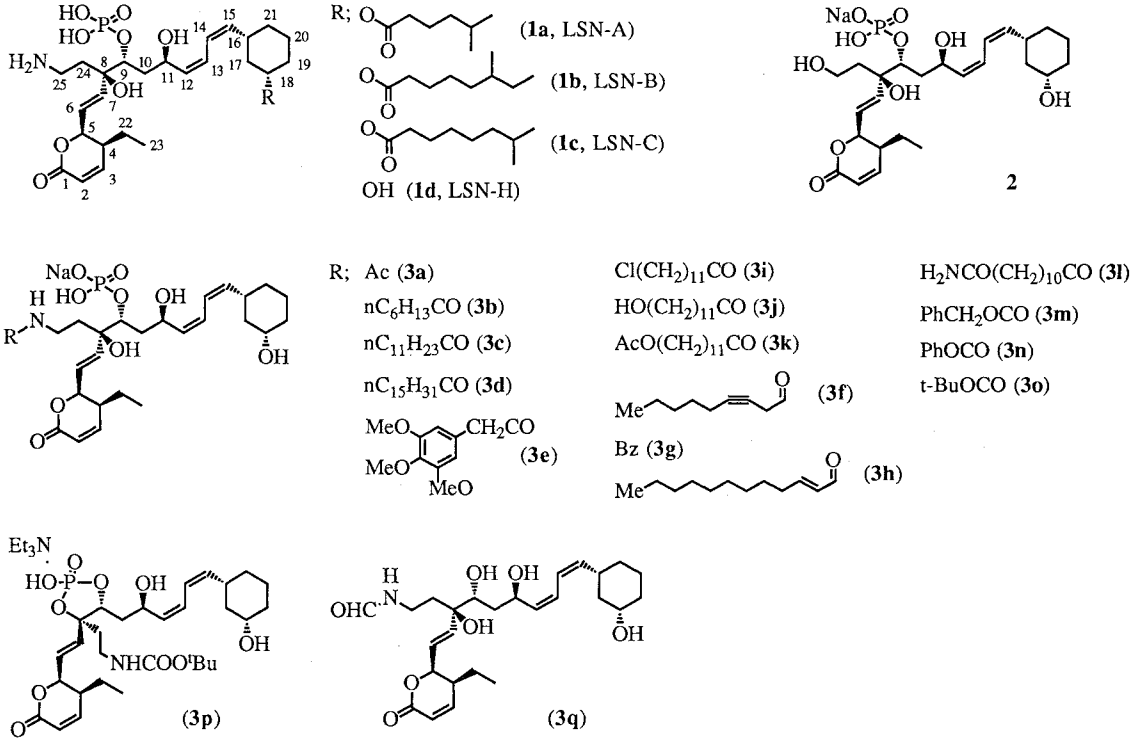

Table 1. Thrombopoietic effects of LSN-H and its derivatives using method A.

Compound	Dose (mg/kg)	% Control	Compound	Dose (mg/kg)	% Control
1d (LSN-H)	1.0	128**	**3f**	1.0	123**
	0.1	150**		0.1	140**
	0.01	104		0.01	110
3a	1.0	134**	**3g**	1.0	toxic
	0.1	187**		0.1	162**
	0.01	154**		0.01	165**
3b	1.0	toxic	**3m**	1.0	121
	0.1	144**		0.1	176**
	0.01	149**		0.01	150**
3c	1.0	toxic	**3n**	1.0	117*
	0.1	118		0.1	126**
	0.01	112		0.01	112
3d	1.0	toxic	**3p**	10.0	146**
	0.1	127		1.0	108
	0.01	175**			
3e	1.0	141**	**3q**	1.0	103
	0.1	154**		0.1	96
	0.01	108		0.01	91

Student t-test (LSN derivative *vs.* control).
** Significant $P<0.01$.
* Significant $P<0.05$.

buffer afforded **3q**.[6]

The thrombopoietic activity of LSN-H was examined using the method described below (method A). LSN-H was dissolved in a physiological saline solution containing 1.25% v/v of aqueous ethanol, and intravenously administrated to ICR mice (female, each 7 weeks old) at 24 hour intervals over a test period of 4 days. Control mice were given only a physiological saline solution containing 1.25% v/v of aqueous ethanol. Seventy two hours after the final administration, blood samples were extracted from the orbit of the animals' eyes and platelet counts taken. The assay was conducted by the electric resistance method using an automatic blood cell counter (K-1000, Toa-Iyo Denshi Co.). The thrombopoietic activities of the LSN-H derivatives were examined using method A (**3a**$\sim$**3g**, **3m**$\sim$**3n**, **3p**$\sim$**3q**) or method B (**2**, **3h**$\sim$**3l**, **3q**) in which LSN-H derivatives were administrated subcutaneously, and blood samples were taken from the heart. The platelet number in the samples were counted using Celltak α (Nihon Koden Co.). The results are shown in Tables 1 and 2.

Interestingly, LSN-H was found to be 100 times less effective than LSN-B *in vitro*, but was found to show comparative thrombopoietic effects *in vivo*. That is, administration of LSN-H (0.1 or 1 mg/kg per day) in mice caused a thrombocytosis comparable to that with LSN-B administration and no detectable levels of cytokines in sera were observed.

A majority of the N-acylated derivatives of LSN-H (**3a**$\sim$**3b**, **3d**$\sim$**3i**, **3k**, **3m**$\sim$**3n**) and alcohol derivative **2** exhibited comparable thrombopoietic effects to that of LSN-H. The cyclic phosphate derivative **3p** also showed thrombopoietic activity although high dose (10 mg/kg) was required. Dephosphorylated derivative **3q**, on the other hand, showed no significant thrombopoietic activity. These results indicate that the amino group is not essential, but the phosphoric acid moiety is essential for thrombopoietic activity.

Table 2. Thrombopoietic effects of LSN-H derivatives using method B

Compound	Dose (mg/kg)	% Control
3h	0.1	157*
	0.01	92
3i	0.1	162*
	0.01	99
3j	0.1	toxic
	0.01	108
3k	0.1	toxic
	0.01	115*
3l	0.1	toxic
	0.01	110
3q	0.1	78*
	0.01	86
2	0.1	toxic
	0.01	157*

Student t-test (LSN derivative *vs.* control).
* Significant $P<0.05$.

Thus an efficient method of preparing LSN-H in large scale has been developed. LSN-H could be a new lead compound for hematological disorders and useful tools for the analysis of regulatory mechanism of hematopoiesis in bone marrow. Further studies on the mechanism of thrombocytosis by LSN-H are now in progress.

Experimental

IR spectra were measured on a JASCO FT/IR-830. NMR spectra were recorded on a Jeol JNM GSX-400 spectrometer. Chemical shifts are reported in ppm (δ) using TMS as an internal standard. FAB-MS were obtained using a Jeol JMS-AX505H. HPLC was carried out under the following conditions: column; Cosmosil 5C18-AR 4.6 x 250 mm (product of Nakarai tesque Inc.), eluting solvent; 20% v/v acetonitrile; 0.5% v/v triethylamine; 79.5% v/v phosphate buffer (pH 3.0), flow rate; 1.0 ml/minute, wavelength; 230 nm. PLE-A was

purchased from Amano Pharm. Co., Ltd. Cosmosil 75C18-OPN (Nakaraitesque Inc.) was used as an adsorbent for column chromatography.

LSN-H (**1d**) and Sodium 25-Deamino-25-hydroxy LSN-H (**2**)

To a solution of the crude oily mixture of LSNs[2] (14.1 g) in 200 ml of acetone and 2 liters of 0.05 M phosphate buffer solution (NaH_2PO_4/Na_2HPO_4, pH = 6.7) was added PLE-A (3.00 g). The resulting mixture was stirred at 37°C. The reaction was monitored by HPLC, and PLE-A was added in three lots of 2.00 g, over a period of 5 days. At the end of this time, the PLE-A was filtered off through Celite and the filtrate washed with EtOAc. The aqueous layer was fractioned and purified by Cosmosil column chromatography using H_2O-MeOH (9:1) as the eluent to afford 1.15 g of **2**. IR (KBr) 3387, 1720 cm^{-1}. ^1H NMR (CD_3OD) δ 0.90~1.21 (6H, m), 1.27~1.94 (10H, m), 2.28 (1H, m), 2.48~2.62 (2H, m), 3.49~3.67 (2H, m), 3.81 (1H, m), 4.19 (1H, m), 4.98~5.11 (3H, m), 5.29 (1H, t, J=9.3 Hz), 5.45 (1H, t, J=9.3 Hz), 5.88 (1H, d, J=16.2 Hz), 6.00~6.10 (2H, m), 6.19~6.34 (2H, m), 7.10 (1H, dd, J=5.2, 9.8 Hz). FAB-MS (m/z) 529 $(M-Na)^-$, 553 $(M+H)^+$, 575 $(M+Na)^+$. HR-FABMS (m/z) Calcd for $C_{25}H_{38}O_{10}PNa_2$ $(M+Na)^+$, 575.1998, Found 575.1984.

After elution of **2**, further elution with H_2O-MeOH (4:1) afforded 2.93 g of LSN-H. IR (KBr) 3391, 1720 cm^{-1}. ^1H NMR (CD_3OD) δ 0.94 (3H, t, J=7.9 Hz), 0.99~1.93 (13H, m), 2.20 (1H, m), 2.50~2.56 (2H, m), 2.99~3.07 (2H, m), 3.53 (1H, m), 4.26 (1H, m), 4.96 (1H, m), 5.09 (1H, m), 5.29 (1H, m), 5.42 (1H, m), 5.91 (1H, d), 5.98 (1H, dd, J=1.0, 9.9 Hz), 6.06 (1H, dd, J=6.6, 15.2 Hz), 6.25 (2H, m), 7.08 (1H, dd, J=5.3, 9.9 Hz). FAB-MS (m/z) 528 $(M-H)^-$, 530 $(M+H)^+$. HR-FABMS (m/z) Calcd for $C_{25}H_{41}NO_9P$ $(M+H)^+$ 530.2519, Found 530.2508.

Typical Procedure for Preparing Sodium N-acyl LSN-H

Sodium N-(12-Acetoxylauroyl) LSN-H (**3k**)

To a solution of *p*-nitrophenyl 12-acetoxylauroate (286 mg) in MeOH (5 ml) was added LSN-H (80 mg). After 4 days of stirring at room temperature, the solvent was evaporated under reduced pressure and the residue obtained was washed with diethyl ether, loaded onto a Cosmosil column and eluted with H_2O-MeOH (1:1) to afford **3k** (67 mg, y. 56%). IR (KBr) 3352, 1725, 1648, 1547 cm^{-1}. ^1H NMR (CD_3OD) δ 0.95 (3H, t, J=7.3 Hz), 0.98~1.20 (3H, m), 1.24~2.00 (29H, m), 2.01 (3H, s), 2.14 (2H, t, J=7.3 Hz), 2.48~2.61 (2H, m), 3.26 (2H, t, J=7.6 Hz), 3.55 (1H, m), 4.06 (2H, t, J=6.6 Hz), 4.25 (1H, m), 4.94 (1H, m), 5.10 (1H, m), 5.30 (1H, m), 5.44 (1H, m), 5.90~6.05 (3H, m), 6.18~6.32 (2H, m), 7.09 (1H, dd, J=5.1, 9.6 Hz). FAB-MS (m/z) 768 $(M-Na)^-$, 792 $(M+H)^+$, 814 $(M+Na)^+$. HR-FABMS (m/z) Calcd for $C_{39}H_{63}NO_{12}PNa_2$ $(M+Na)^+$ 814.3883, Found 814.3877. *Anal* Calcd for $C_{39}H_{63}NO_{12}PNa \cdot 2H_2O$: C 56.58, H 8.16, N 1.69, P 3.74, Na 2.78. Found: C 56.66, H 7.89, N 1.77, P 3.63, Na 2.64.

References

1) KOHAMA, T.; R. ENOKITA, T. OKAZAKI, H. MIYAOKA, A. TORIKATA, M. INUKAI, I. KANEKO, T. KAGASAKI, Y. SAKAIDA, A. SATOH & A. SHIRAISHI: Novel microbial metabolites of the phoslactomycines family induce production of colony-stimulating factors by bone marrow stromal cells. I. Taxonomy, fermentation and biological properties. J. Antibiotics 46: 1503~1511,1993

2) KOHAMA, T.; T. NAKAMURA, T. KINOSHITA, I. KANEKO & A. SHIRAISHI: Novel microbial metabolites of the phoslactomycines family induce production of colony-stimulating factors by bone marrow stromal cells. II. Isolation, physico-chemical properties and structure determination. J. Antibiotics 46: 1512~1519, 1993

3) ISHIBASHI, T.; H. KIMURA, Y. SHIKAMA, T. UCHIDA, S. KARIYONE, T. HIRANO, T. KISHIMOTO, F. TAKATSUKI & Y. AKIYAMA: Interleukin-6 is a potent thrombopoietic factor *in vivo* in mice. Blood 74: 1241~1244, 1989

4) YONEMURA, T.; M. KAWAKITA, T. MASUDA, K. FUJIMOTO & K. TAKATSUKI: Effect of recombinant humane interleukin-11 on rat megakaryopoiesis and thrombopoiesis *in vivo*: Comparative study with interleukin-6. Br. J. Haematol. 84: 16~23, 1993

5) KOHAMA , T.; H. MAEDA, J. I. SAKAI, A. SHIRAISHI & K. YAMASHITA: Leustroducsin B, a new cytokine inducer derived from an actinomycetes, induces thrombocytosis in mice. J. Antibiotics 49 (1): 1996, in press

6) SHIBATA, T.; S. KURIHARA, K. YODA & H. HARUYAMA: Absolute configuration of leustroducsin H. Tetrahedron 51: 11999~12012, 1995