Futalosine and Its Derivatives, New Nucleoside Analogs

Nobuo HOSOKAWA,*[,a] Hiroshi NAGANAWA,[a] Takako KASAHARA,[a] Seiko HATTORI,[a] Masa HAMADA,[a] Tomio TAKEUCHI,[a] Shinobu YAMAMOTO,[b] Kayoko S. TSUCHIYA,[b] and Makoto HORI[b]

Institute of Microbial Chemistry,[a] 3–14–23 Kamiosaki, Shinagawa-ku, Tokyo 141–0021, Japan and Showa College of Pharmaceutical Sciences,[b] 3–3156, Higashi-Tamagawagakuen, Machida, Tokyo 194–8543, Japan.
Received February 3, 1999; accepted March 23, 1999

Futalosine, a new nucleoside analog, was isolated from a fermentation broth of *Streptomyces* sp. MK359-NF1. Some chemical derivatives of futalosine were prepared. 6-*O*-Methylfutalosine methylester inhibited growth of HeLa-S3 cells *in vitro* (IC_{50}=19.5 μg/ml) in contrast to the weak activity of futalosine. 6-*O*-Methylfutalosine methylester at concentrations higher than 10 μg/ml inhibited incorporation of ^{3}H-TdR and ^{3}H-UR but not ^{3}H-Leu into the acid-soluble fractions of HeLa-S3 cells.

Key words futalosine; nucleoside analog; HeLa-S3

Experimental

Physicochemical Properties of Futalosine (1) $[\alpha]_D^{22}$ +1.57° (c=0.40, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3200, 1700, 1605, 1560, 1395. UV (H_2O) λ_{max} nm (log ε): 245 (3.96). HR-FAB-MS (*m/z*): 413 $(M-H)^-$, Found 413.1122, Calcd 413.1097 ($C_{19}H_{17}N_4O_7$).

Preparation of 6-*O*-Methylfutalosine Methylester (2) To prepare the compound **2**, 16 mg (38 μmol) of **1** was dissolved in MeOH (1.0 ml) and mixed with an excess of trimethylsilyldiazomethane (Me_3SiCHN_2) in diethylether. After the reaction mixture was kept at 24 °C for 2 h, the solution was concentrated *in vacuo*, the residue was dissolved in MeOH (0.5 ml) and the solution was subjected to preparative HPLC. 1-*N*-Methylfutalosine methylester was eluted at a retention time of 20 min, while **2** was eluted at a retention time of 50 min. By evaporation *in vacuo*, **2** was obtained as a colorless powder (4.0 mg, 25% yield).

Physicochemical Properties of 2 $[\alpha]_D^{22}$ +13.6° (c=0.22, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1730, 1695, 1605. UV (MeOH) λ_{max} nm (log ε): 240 (4.16), 285 (3.12). HR-FAB-MS (*m/z*): 443 $(M+H)^+$, Found 443.1588, Calcd 443.1576 ($C_{21}H_{23}N_4O_7$). ^{1}H-NMR (in CD_3OD) δ: 4.17 (3H, s), 3.92 (3H, s), ^{13}C-NMR (in CD_3OD) δ: 54.81 (-OCH_3), 162.23 (C-6), 52.88 (methylester), 167.59 (C-14).

Determination of [^{3}H]-TdR, [^{3}H]-UR and [^{3}H]-Leu in Acid-Soluble and Acid-Insoluble Fractions of Cells Cells were seeded at a density of 2.0×10^4 cells/ml medium/2 cm^2 well and incubated at 37 °C for a day. Then, the culture medium was removed, 0.5 ml/well of the serum free medium including a test compound was added, the cultures were incubated at 37 °C for 20 min, and thereafter labeled with 25 μCi/well each of [^{3}H]-TdR, [^{3}H]-UR, or [^{3}H]-Leu at 37 °C for 60 min. The medium was removed, the cell layers were washed three times with 1 ml/well of cold phosphate buffered saline (PBS) (−), 0.5 ml/well of cold 5% trichloroacetic acid (TCA) was added, the mixtures were kept cool for 40 min, and the supernatant (acid-soluble fraction) was taken. The remainder was washed once with 1 ml/well of 5% TCA and dissolved in 0.5 ml/well of 0.5 N KOH by allowing it to stand at a room temperature for 40 min (acid-insoluble fraction).

Analytical Procedures An HPLC system (3520, SSC Co., Ltd., Tokyo) using a column (ODS-2151-A, 6$\phi\times$150 mm, SSC Co., Ltd.) was developed with 30% MeOH/12 mM phosphate buffer (pH 2.4) at a flow rate of 2 ml/min at 40 °C, and the elution was monitored at 250 nm. **1** and **2** was eluted at a retention time of 7 and 18 min, respectively. *Rf* values on TLC (Kieselgel 60 F_{254} Art, 5715, Merck) of **1** were 0.57 (*n*-PrOH : H_2O : 1 N NH_4OH= 10 : 1 : 1) and 0.48 (*n*-BuOH : AcOH : H_2O=4 : 1 : 1). The *Rf* values of **2** were 0.61 ($CHCl_3$: MeOH=5 : 1) and 0.57 (*n*-BuOH : MeOH : H_2O=5 : 1 : 2), and the spots on the TLC were detected with phosphomolybdate-H_2SO_4.

Preparative HPLC A reverse-phase column (Capcell Pack C_{18}, 5 μm-100 Å, 20×150 mm, Shiseido Co., Ltd., Japan) was used for separation employing the same conditions as the analytical HPLC system, except that the flow rate has 9.9 ml/min.

General UV spectra were recorded on a Hitachi U-3210 spectrophotometer and IR spectra on a Hitachi 260-10 spectrophotometer. NMR spectra were recorded on a JEOL-A500 NMR spectrometer at 500 MHz for ^{1}H- and at 125 MHz for ^{13}C-NMR. Mass spectra were measured on a JEOL JMS-SX 102 mass spectrometer. Optical rotation was measured with a Perkin-Elmer 241 polarimeter.

Results and Discussion

The futalosine producing strain, *Streptomyces* sp. MK359-NF1 was cultured by shaking at 27 °C for 7 d in 500-ml Erlenmeyer flasks each containing 110 ml of a medium composed of 3% starch, 0.2% yeast extract, 1.5% soybean meal, 0.3% NaCl, 0.05% $MgSO_4\cdot7H_2O$, 0.03% $CaCO_3$, 0.0005% $CuSO_4\cdot5H_2O$, 0.0005% $MnCl_2\cdot4H_2O$, 0.0005% $ZnSO_4\cdot7H_2O$ and water to volume (pH 7.0 before sterilization). Futalosine was isolated from the fermentation broth according to the procedures as shown in Fig. 1.

The UV absorption maximum of futalosine (**1**) at 245 nm (log ε 3.96) in H_2O suggested the presence of a hypoxanthine chromophore in the molecule. The molecular formula of **1**

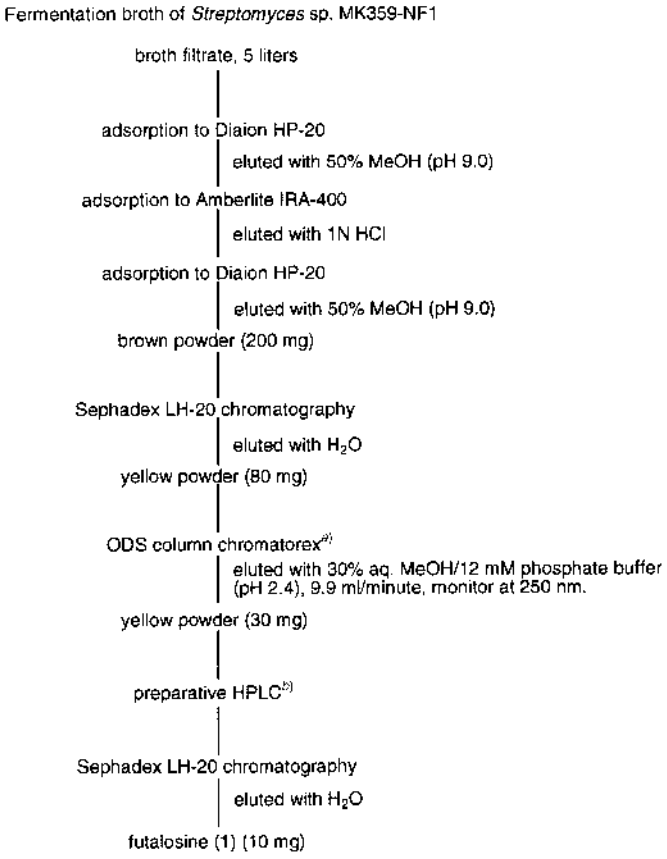

Fig. 1. Isolation Procedures for Futalosine

a) Chromatorex®, 2×30 cm, SSC Co., Ltd., Japan. *b*) See preparative HPLC.

* To whom correspondence should be addressed.

Table 1. NMR Spectrum Data of **1** in D_2O

Carbon No.	$^{13}C^{a)}$	$^{1}H^{b)}$
2	146.29 (d)	7.92 (1H, s)
4	149.12 (s)	—
5	124.62 (s)	—
6	158.93 (s)	—
8	140.84 (d)	8.09 (1H, s)
1′	88.82 (d)	5.86 (1H, d, 5.0)
2′	73.62 (d)	4.88 (1H, t, 5.1)
3′	73.54 (d)	4.42 (1H, t, 5.2)
4′	84.61 (d)	4.24 (1H, q, m)
5′	28.27 (t)	2.22, 2.35 (2H, m)
6′	34.99 (t)	3.08, 3.12 (2H, m)
7′	205 (s)	—
8′	136.95 (s)	—
9′	129.47 (d)	8.17 (1H, br s)
10	133.24 (s)	—
11	134.62 (d)	8.02 (1H, d, 7.8)
12	129.47 (d)	7.40 (1H, t, 7.8)
13	132.15 (d)	7.79 (1H, d, 7.7)
14	172.0 (s)	—

a) Measured at 125 MHz; chemical shifts in ppm (dioxane; δ_C=67.4). *b*) Measured at 500 MHz; chemical shifts in ppm (D_2O; δ_H=4.8).

was established as $C_{19}H_{18}N_4O_7$ (MW 414) by HR-FAB-MS [Obs. *m/z* 413.1122, $(M-H)^-$: Calcd For $C_{19}H_{17}N_4O_7$, *m/z* 413.1097]. The ^{1}H- and ^{13}C-NMR data (Table 1) and heteronuclear multiple bond correlation (HMBC) experiment showed that futalosine was an analog of a nucleoside which was coupled to an isophthalic acid moiety. Analyses of the ^{1}H–^{1}H correlated spectroscopy (COSY) and HMBC spectra of **1** indicated that a methylene (δ_H 3.08, 3.12) at the C-6′ position in the nucleoside was directly linked to one carbonyl group of the isophthalic acid, and that the other carboxyl group was free (Fig. 3). The ^{1}H-NMR spectrum of **1** in dimethyl sulfoxide (DMSO)-d_6 showed no signals arising from exchangeable protons at C-6, N-1, 2′ or 3′ in the nucleoside moiety or from the carboxyl group (C-14) in the isophthalic acid moiety. In order to confirm the structure of **1**, a methyl derivative (**2**) was prepared by the treatment of **1** with trimethylsilyldiazomethane. The NMR spectral data of **2** and 1-*N*-methylfutalosine methylester, which was obtained together with **2** by methylation of **1** in DMSO-d_6 (data not shown) supported the presence of the hypoxanthine ring and the free carboxyl group at C-14 of **1**. The NMR spectra of a diacetyl derivative which was prepared by the treatment of **1** with acetic anhydride and pyridine revealed the presence of the hydroxyl groups at 2′ and 3′ positions. Moreover, difference nuclear Overhauser effects (NOEs) were observed between H-8 and H-1′, H-8 and H-2′, and H-2′ and H-3′ of **1** in D_2O. This NOE data suggested the hydroxyl groups at 2′ and 3′ were attached to the same side in the sugar moiety. The coupling constants of the sugar moiety in **1** were similar to those of the ribose moiety in oxanosine.[1)] But the stereochemistry of the sugar moiety remained to be determined. Thus, the planar structure of futalosine was determined as shown in Fig. 2.

Compound **2** inhibited the growth of HeLa-S3 (IC_{50}= 19.5 μg/ml) *in vitro* in contrast to the weak activity of futalosine (IC_{50}>100 μg/ml). Cell growth was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method and the inhibition caused by the samples was calculated as reported.[2)] **2** at 10 μg/ml inhibited incorporation of ^{3}H-thymidine and ^{3}H-uridine but not ^{3}H-leucine into the acid-soluble fraction of HeLa-S3 cells, over a 60 min labeling period (Fig. 4). **1** in contrast, hardly inhibited radiolabel incorporation even at a concentration as high as 100 μg/ml. The difference in the effect between the two compounds may be a reflection of their different affinities to nucleoside transporters,[3)] although an effect on other intracellular metabolic processes is possible. Incorporation into the acid-insoluble fraction of ^{3}H-thymidine and ^{3}H-uridine were also inhibited by **2**, which may reflect the lowered radioactivities in the acid-soluble pools in the cells treated with **2**. Some inhibition by **2** of the incorporation ^{3}H-leucine into the acid-insoluble fraction, accompanied by the corresponding enhancement of the incorporation into the acid-soluble pool (134% of the control) may be due to inhibition of the polymerization step of protein synthesis. Additional experiments also showed that **2** was not a simple inhibitor of the nucleoside transporter; dipyridamole, an inhibitor of this process, was antagonistic to FUdR while **2** was rather synergistic to FUdR (data not shown). Furthermore, the first 20 s labeling *in vitro* of HeLa-S3 cells with ^{3}H-TdR at 25 °C (regarded as the membrane transport of the nucleoside) was inhibited by **2** only 1/10 as strongly as was inhibited by dipyridamole when each was compared at 50% growth-inhibitory concentration for HeLa-S3 cells (data not shown).

No sign of acute toxicity for **1** and **2** was detectable at 150 mg/kg in mice (i.v.). **1** and **2** did not inhibit *in vitro* growth of any bacteria, fungi or yeast at 100 μg/ml.

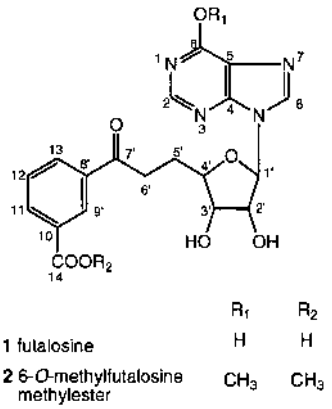

Fig. 2. Structures of **1** and **2**

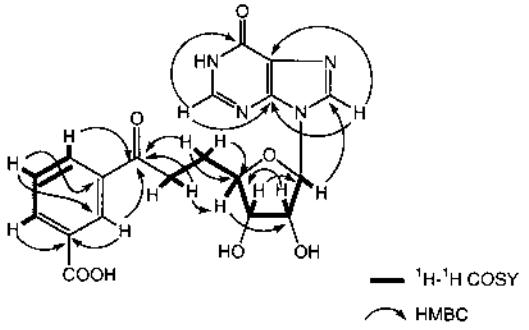

Fig. 3. Structure of **1** Elucidated by NMR Analyses

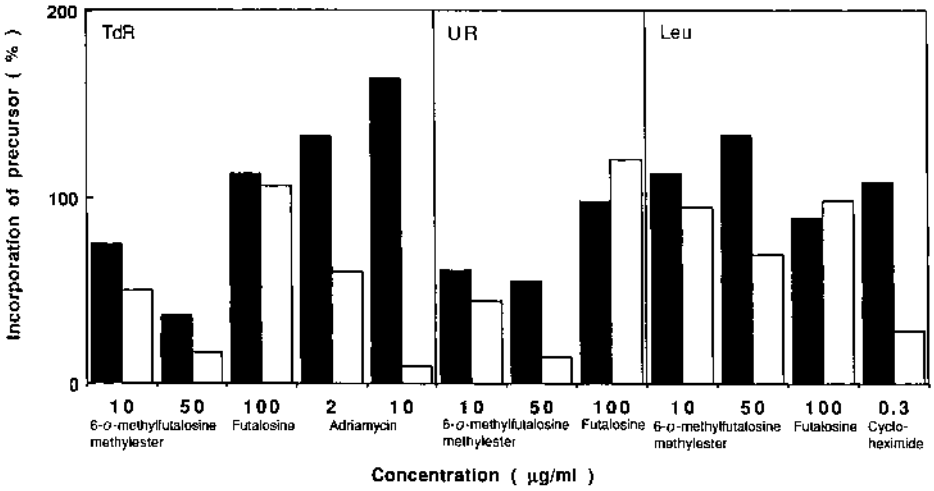

Fig. 4. Effect of 6-*O*-Methylfutalosine Methylester on Incroporation of [^{3}H]-Thymidine, -Uridine and -Leucine in Acid-Soluble and Acid-Insoluble Fractions of HeLa-S3 Cells

A test compound dissolved in 0.5 ml of serum-free medium was added to 3×10^4 cells (one-day precultured) in a 2 cm^2-well. After standing at 37 °C for 20 min, the culture received 20 μl solution of either ^{3}H-TdR, -UR or -Leu (0.25 μCi each) and was kept another 60 min at 37 °C. Labeling was terminated by removal of the radioactive medium and washing the cell layers with cold PBS (−). 0.5 ml of cold 5% TCA was added to each well and the labeled cells were fractionated into acid-soluble and -insoluble fractions, whose radioactivities were measured. Closed bars and open bars stand for acid-soluble and -insoluble fractions, respectively. Radioactivities of the control run were taken as 100%. Adriamycin and cycloheximide (inhibitors of the polymerization steps of DNA synthesis and protein synthesis, respectively) were included in the experiment for comparison.

Acknowledgements We are grateful to Mr. Nariaki Takao, Miss Futaba Shimizu and Miss Sachiko Miyata for helpful technical assistance.

References

1) Shimada N., Yagisawa N., Naganawa H., Takita T., Hamada M., Takeuchi T., Umezawa H., *J. Antibiot*., **34**, 1216—1218 (1981).
2) Tuchiya K. S., Kanbe T., Hori M., Uehara Y., Takeuchi T., *Biol. Pharm. Bull*., **16**, 908—911 (1993).
3) Mark G., Nick B., Sylvia Y. M. Y., Manickavasaga S., Christine E. B., Anthony D., Francis Y. P. K., Imogen C., Carol E. C., James D. Y., Stephen A. B., *Nature Medicine*, **3**, 89—93 (1997).