Tryprostatins A and B, Novel Mammalian Cell Cycle Inhibitors Produced by *Aspergillus fumigatus*

Sir:

In the course of our screening for new inhibitors of mammalian cell cycle[1], we have discovered two novel compounds named tryprostatins A (**1**) and B (**2**) (Fig. 1), from the secondary metabolites of a marine fungal strain BM939. The producing strain was isolated from a sea sediment sample collected in the sea bottom (760 meters deep) of the mouth of Oi river, Sizuoka prefecture, Japan, and was identified as *Aspergillus fumigatus* through a taxonomic study. Here we preliminarily report the isolation, structures and biological activities of **1** and **2**.

The producing strain was cultured in a 30-liter jar fermenter containing 18 liters of fermentation medium (glucose 3%, soluble starch 2%, soybean meal 2%, K_2HPO_4 0.5% and $MgSO_4 \cdot 7H_2O$ 0.05%, adjusted at pH 6.5 before sterilization) containing 0.05% of CA-123 and KM-68 antifoam, respectively. The fermentation was carried out for 72 hours at 28°C under the following condition; 350 rpm stirring speed and 7 liters/minute aeration rate.

The fermentation and the following separation procedure were monitored by the inhibitory activity on the cell cycle progression of a mouse tsFT210 cell line which is a temperature-sensitive $p34^{cdc2}$ mutant. The cells were growing normally at 32°C, but arrested in the G2 phase at 39°C[1~3].

The cultured whole broth was centrifuged to separate

Fig. 1. Structures of tryprostatins A (**1**) and B (**2**).

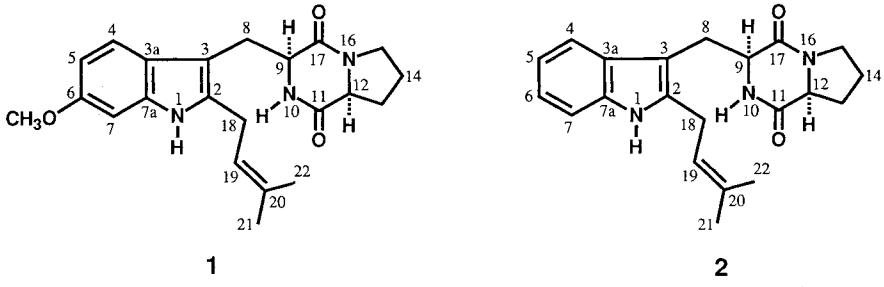


Fig. 2. Purification procedure for tryprostatins A (**1**) and B (**2**).

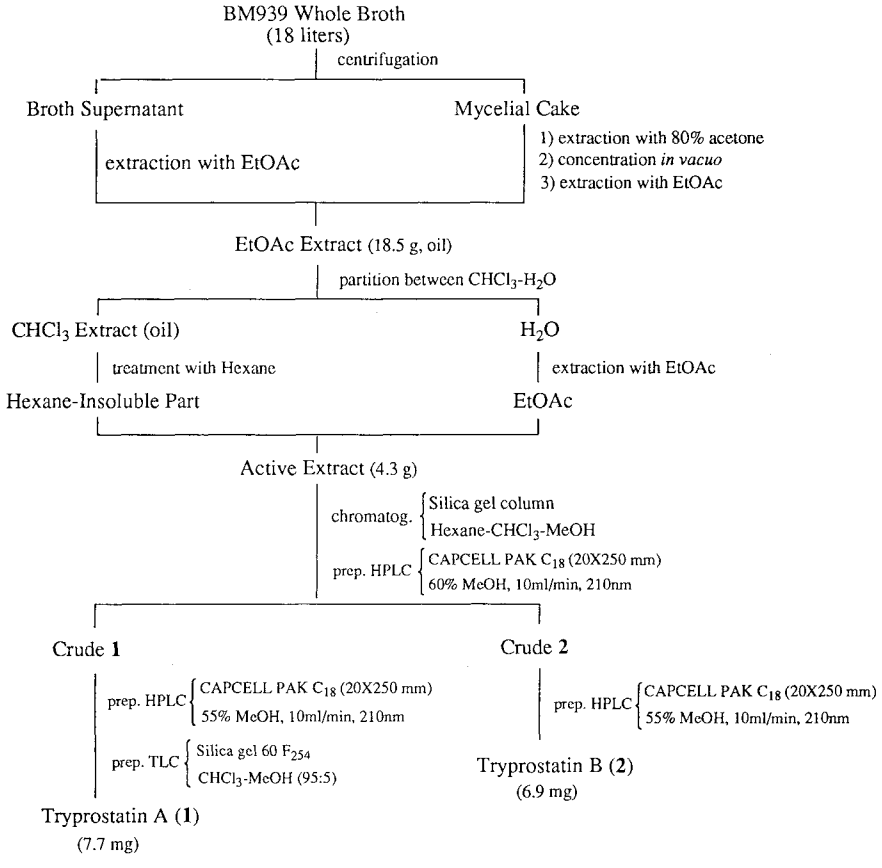


Table 1. Physico-chemical properties of tryprostatins A (**1**) and B (**2**).

Characteristics	1	2
Appearance	Pale yellow crystalline solid	Pale yellow crystalline solid
MP	120-123°C	102-105°C
$[\alpha]_D^{27}$	-69.7°(c 0.70, $CHCl_3$)	-71.1°(c 0.63, $CHCl_3$)
Molecular Formular	$C_{22}H_{27}N_3O_3$	$C_{21}H_{25}N_3O_2$
HR-EI-MS	M^+	M^+
Found (*m/z*)	381.2050	351.1943
Calcd (*m/z*)	381.2050	351.1944
UV λ_{max}^{MeOH} nm (ε)	227 (24540) 270 (5450) 297 (6590)	226 (23780) 277 (8690) 298 (sh, 7180)
IR ν_{max}^{KBr} cm^{-1}	3365, 3270, 3070, 2965, 2930, 2880, 2860, 1670, 1655, 1460, 1430, 1160	3370, 3270, 3070, 2970, 2930, 2875, 2860, 1670, 1655, 1460, 1435, 1160

Fig. 3. UV spectra of tryprostatins A (**1**) and B (**2**).

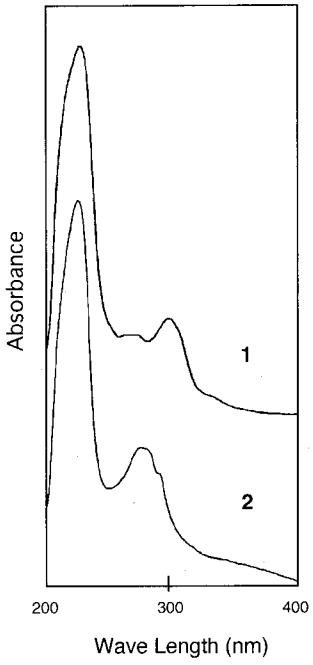
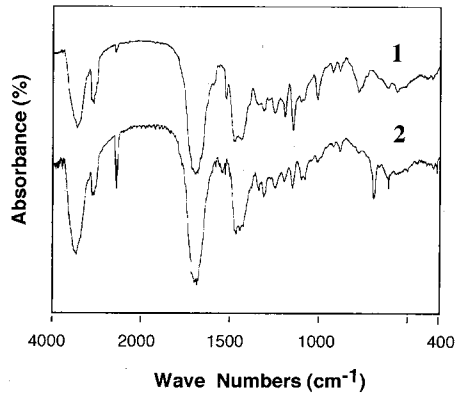


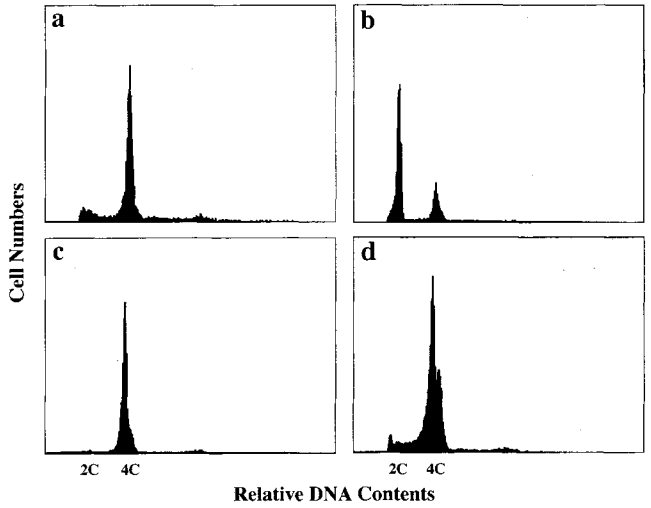
Fig. 4. IR spectra of tryprostatins A (**1**) and B (**2**).



to a broth supernatant (18 liters) and a mycelial cake. The latter was extracted with 80% aqueous acetone which was evaporated *in vacuo* to remove acetone. Both the broth supernatant and the mycelium extract showed an inhibitory activity on the cell cycle progression of tsFT210 cells and thus both were extracted respectively with the same volume of EtOAc. The EtOAc solution obtained was combined and concentrated *in vacuo* to afford an oily extract (18.5 g) which was further purified as shown in Fig. 2 to give an active extract (4.3 g). This extract was then separated by a combination of column chromatography (silica gel 60, Merck), repeated HPLC (CAPCELL PAK C_{18}, Shiseido) and preparative TLC (silica gel 60 F_{254} plate, Merck) to obtain **1** (7.7 mg) and **2** (6.9 mg) (Fig. 2).

Tryprostatins A (**1**) and B (**2**) were obtained both as pale yellowed crystalline solids. The physico-chemical properties of **1** and **2** are summarized in Table 1. Their molecular formulae, $C_{22}H_{27}N_3O_3$ for **1** and $C_{21}H_{25}N_3O_2$ for **2**, were determined by HR-EI-MS measurements (Found *m/z* 381.2050 (M^+), Calcd for $C_{22}H_{27}N_3O_3$ 381.2050 for **1**; Found *m/z* 351.1943 (M^+), Calcd for $C_{21}H_{25}N_3O_2$ 351.1944 for **2**). The UV spectra (Fig. 3) of **1** and **2** respectively revealed the presence of a 6-*O*-methylindole chromophore in **1** with the absorption maxima at 227 (ε 24540), 270 (5450) and 297 nm (6590)[4] and the presence of an indole chromophore in **2** with 226 (23780), 277 (8690) and 298 (sh, 7180)[5]. And their IR spectra closely resembled in the functional group region, as shown in Fig. 4. The multiple absorption at

Fig. 5. Effects of **1** and **2** on cell cycle progression of tsFT210 cells.



The tsFT210 cells were synchronized in the G2 phase by incubation at 39.4°C for 17 hours at a density of 2×10^5 cells/ml in RPMI-1640 medium supplemented with 5% calf serum. Then, the cells were maintained at 39.4°C up to 4 hours (a) or allowed to progress into mitosis at 32°C for 4 hours in the absence (b) or in the presence of tryprostatin A (50 μg/ml) (c) and tryprostatin B (12.5 μg/ml) (d), respectively.

1670 and 1655 cm^{-1} due to amide groups together with the absence of the amide II band near 1550 cm^{-1} suggest the presence of diketopiperazine system[5~7] in both **1** and **2**. Eventually, the structures of tryprostatins A and B were determined as shown in Fig. 1 by detailed analyses of their ^1H and ^{13}C NMR spectra with the aid of 2D NMR techniques including field-gradient heteronuclear multiple-bond correlation (FG-HMBC) spectroscopy.

The molecules of **1** and **2** are composed from a 2-isoprenyltryptophan moiety and a proline residue, forming a diketopiperazine unit, which are distinguished from the molecules of fumitremorgin series[8] in the respect of opening the centered heterocyclic ring at C–N bond between 18 and 10 positions. Only few kinds of natural products structurally related to **1** and **2** such as deoxybrevianamide-E had so far been reported[5], and present result provides the first example of natural product belonging to this novel class as the inhibitor of mammalian cell cycle. Tryprostatins A and B may be shunt metabolites of verruculogen[8] and fumitremorgin series[8] like as deoxybrevianamide-E in biogenesis of brevianamides[7].

Tryprostatins A (**1**) and B (**2**) completely inhibited cell cycle progression of tsFT210 cells in the G2/M phase at final concentrations of 50 μg/ml of **1** and 12.5 μg/ml of **2**, respectively (Fig. 5). Further studies on their biological activities and mechanism of their action are currently undertaken.

Details of the structural and biological studies will be reported elsewhere.

Acknowledgments

We thank Ms. TAMIKO CHIJIMATSU (RIKEN) for the measurements of FG-HMBC spectra. This research was supported in part by a Grant from the Ministry of Education, Science and Culture, Japan and a Grant for "Biodesign Research Program" from RIKEN (C.-B. C. and H. O.).

CHENG-BIN CUI
HIDEAKI KAKEYA
GEN OKADA
RIE ONOSE
MAKOTO UBUKATA[†]
ISAMU TAKAHASHI[††]
KIYOSHI ISONO[††]
HIROYUKI OSADA*

The Institute of Physical and Chemical Research (RIKEN),
Wako, Saitama 351-01, Japan
[†]Biotechnology Research Center, Toyama Prefectural University,
Kosugi-machi, Imizu-gun, Toyama 939-03, Japan
[††]Central Research Laboratories
Kaken Pharmaceutical Co Ltd.,
Gensuke, Fujieda, Shizuoka 426, Japan

(Received July 20, 1995)

References

1) OSADA, H.; F. HANAOKA, R. ONOSE, M. INAGAKI & K. ISONO: Screening of cell cycle inhibitors from microbial metabolites by a bioassay using a mouse cdc2 mutant cell line, tsFT210. submitted to J. Antibiotics 1995
2) MINEO, C.; Y. MURAKAMI, Y. ISHIMI, F. HANAOKA & M.-A. YAMADA: Isolation and analysis of a mammalian temperature-sensitive mutant defective in G2 functions. Exp. Cell Res. 167: 53~62, 1986
3) TH'NG, J. P. H.; P. S. WRIGHT, J. HAMAGUCHI, M. G. LEE, C. J. NORBURY, P. NURSE & E. M. BRADBURY: The FT210 cell line is a mouse G2 phase mutant with a temperature-sensitive *CDC2* gene product. Cell 63: 313~324, 1990
4) FAYOS, J.; D. LOKENSGARD, J. CLARDY, R. J. COLE & J. W. KIRKSEY: Structure of verruculogen, a tremor producing peroxide from *Penicillium verruculosum*. J. Am. Chem. Soc. 96: 6785~6787, 1974
5) STEYN, P. S.: The structures of five diketopiperazines from *Aspergillus ustus*. Tetrahedron 29: 107~120, 1973
6) STEYN, P. S.: Austamide, a new toxic metabolite from *Aspergillus ustus*. Tetrahedron Lett. 1971: 3331~3334
7) BRICH, A. J. & J. J. WRIGHT: Studies in relation to biosynthesis-XLII. The structural elucidation and some aspects of the biosynthesis of the brevianamide-A and -E. Tetrahedron 26: 2329~2344, 1970
8) STEYN, P. S. & R. VLEGGAAR: Tremorgenic mycotoxins. Progress in the Chemistry of Organic Natural Products (Founded by L. ZECHMIESTER, Edited by W. HERZ, H. GRISEBACH and G. W. KIRBY). 48: 1~80, 1985