

COMMUNICATIONS TO THE EDITOR

**Spirotryprostatin B, a Novel Mammalian
Cell Cycle Inhibitor Produced
by *Aspergillus fumigatus***

Sir:

In previous papers¹⁻³, we reported three new natural diketopiperazines, tryprostatins A, B and demethoxy-fumitremorgin C together with fumitremorgin C, 12,13-dihydroxyfumitremorgin C, fumitremorgin B and ver-ruculogen, as a new group of M-phase inhibitors of the mammalian cell cycle, which were isolated from the secondary metabolites of a fungus *Aspergillus fumigatus*. In order to obtain larger amounts of those compounds to examine their biological activities and mechanism of action in detail, we carried out a large scale fermentation of the producing strain and from the fermentation broth, larger quantity of the former four compounds were prepared. In the course of the preparation, we have now isolated a novel compound named spirotryprostatin B (**1**) which inhibited the cell cycle progression of tsFT210 cells at the G2/M phase. In this communication, we report the isolation, structure and biological activities of **1**.

The producing strain was cultured in a 600-liter jar fermenter containing 400 liters of fermentation medium (glucose 3%, soluble starch 2%, soybean meal 2%, K₂HPO₄ 0.5% and MgSO₄·7H₂O 0.05%, adjusted to pH 6.5 before sterilization) containing 0.05% of CA-123 and KM-68 antifoam, respectively. The fermentation was carried out for 66 hours at 28°C under the following condition; 350 rpm stirring speed and 200 liters/minute aeration rate.

The whole broth was filtered to separate to a broth supernatant (370 liters) and a mycelial cake. The latter was extracted with 90% aqueous acetone which was evaporated *in vacuo* to remove acetone. Both the broth supernatant and the mycelium extract (60 liters) were extracted respectively with EtOAc. The EtOAc solution obtained was combined and concentrated *in vacuo* to afford an oily extract (1.2 liters) which was further

Fig. 1. Structure of spirotryprostatin B (**1**).

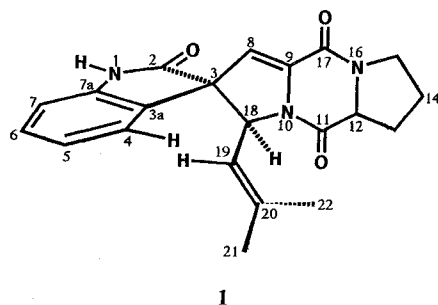


Fig. 2. Isolation procedure for spirotryprostatin B (**1**).

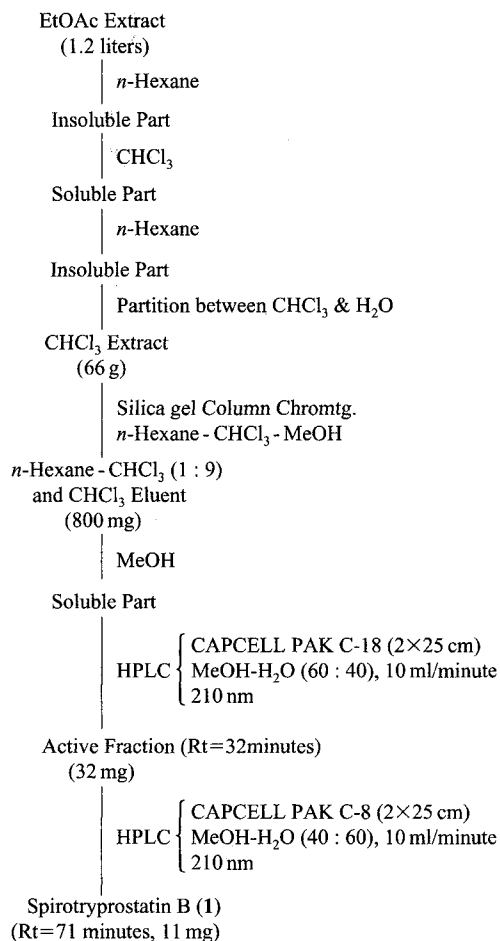


Fig. 3. UV spectrum of spirotryprostatin B (**1**) in MeOH solution.

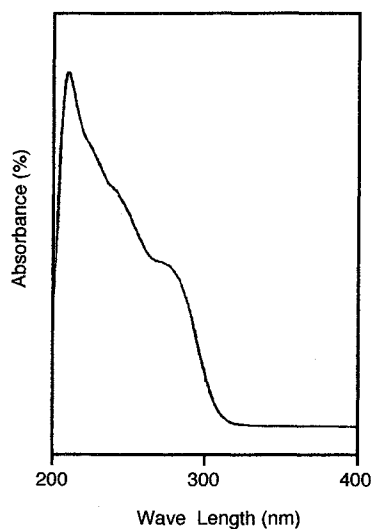
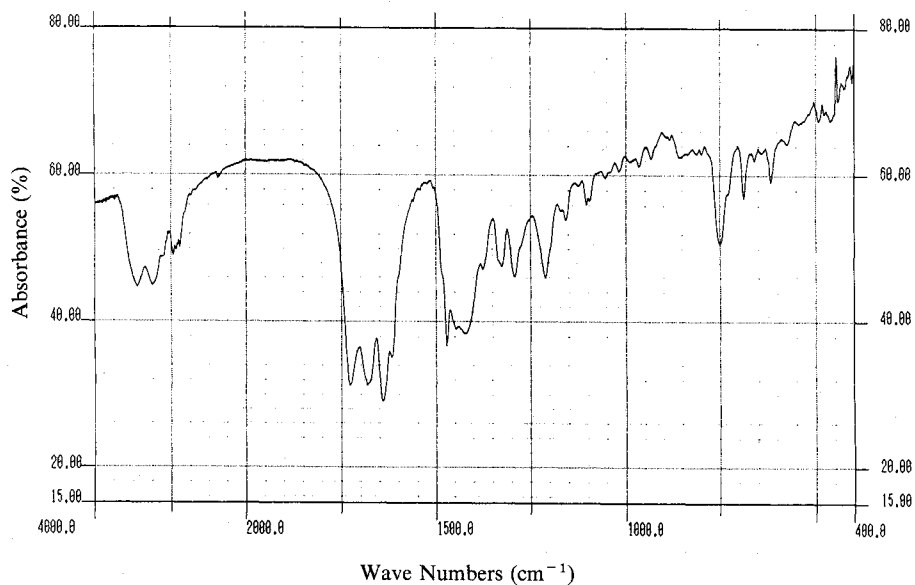
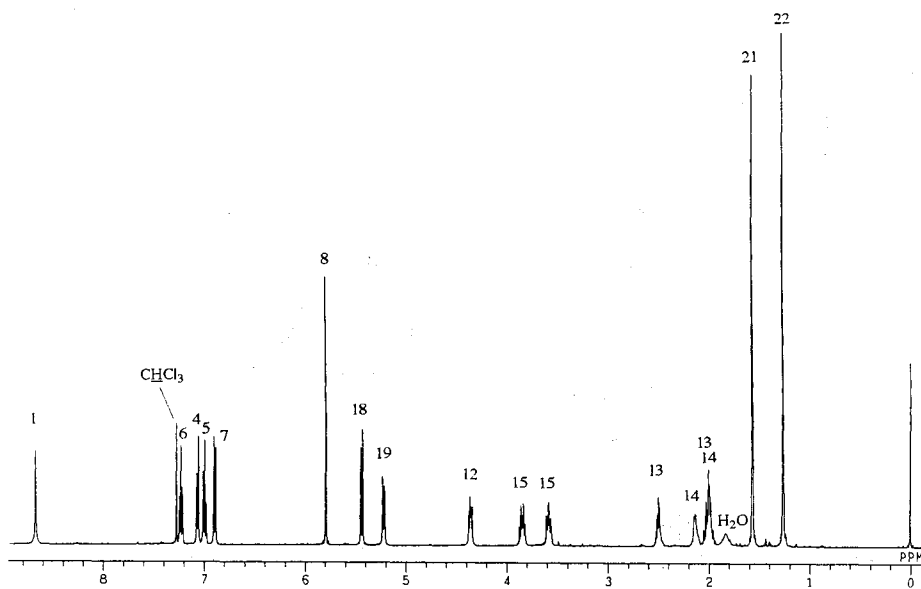
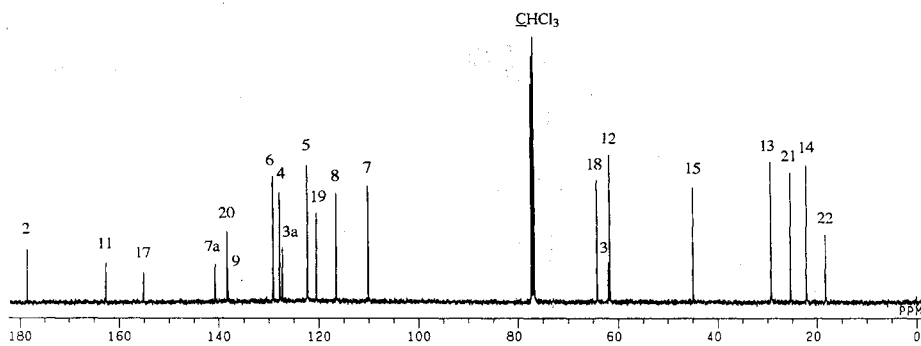


Fig. 4. IR spectrum of spirotryprostatin B (I) on a KBr disk.

Fig. 5. 500 MHz ¹H NMR spectrum of spirotryprostatin B (I) in chloroform-*d*.Fig. 6. 125 MHz ¹³C NMR spectrum of spirotryprostatin B (I) in chloroform-*d*.

purified as shown in Fig. 2 to give an active extract (66 g). This extract was then separated by a combination of column chromatography (silica gel 60, Merck) and repeated HPLC (CAPCELL PAK C-18 and CAPCELL PAK C-8, Shiseido) to give 11 mg of **1** (Fig. 2).

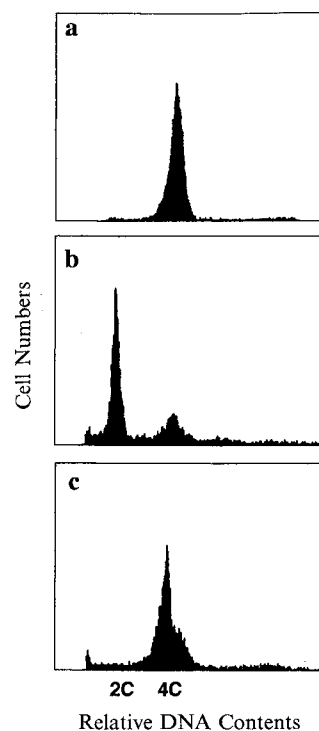
The separation procedure for **1** was monitored by the inhibitory activity on the cell cycle progression of mouse tsFT210 cells which have a temperature-sensitive p34^{cdc2} and the cells were growing normally at 32°C, but arrested in the G2 phase at 39°C^{4,5}. The bioassay was carried out by the synchronously cultured assay as we have previously reported².

Spirotryprostatin B (**1**) was obtained as a slightly yellow-colored crystalline solid having melting point 137~138°C and showed $[\alpha]_D^{22} - 162.1^\circ$ (*c* 0.92, CHCl₃). Its molecular formula was determined to be C₂₁H₂₁N₃O₃ by HR-EI-MS measurement (Found *m/z* 363.1612 (M⁺), Calcd for C₂₁H₂₁N₃O₃ 363.1609). In the UV spectrum, **1** showed a unique absorption curve with absorption maxima at 212 (ϵ 36660), 227 (sh, 28820), 242 (sh, 24830), 272 (sh, 17350) and 286 nm (sh, 14810) and the IR spectrum of **1** showed the absorption at 3440, 3240 (N-H), 1730 (γ -lactam C=O), 1680, 1655 (amide C=O) and 1640 cm⁻¹ (C=C) in the functional group region. The UV and IR spectra of **1** are shown in Figs. 3 and 4, respectively. The amide carbonyl absorption at 1680 and 1655 cm⁻¹ together with the absence of the amide II band near 1550 cm⁻¹ in the IR spectrum suggested the presence of a diketopiperazine system^{6~8}) in **1**. The carbonyl absorption at 1730 cm⁻¹ could be assigned, after structural elucidation by NMR studies, to the carbonyl of the γ -lactam moiety⁹) which fused to a benzene ring in **1**.

The ¹H and ¹³C NMR spectra of **1** are given in Figs. 5 and 6, respectively, which were exactly assigned by means of 2D NMR techniques including pulse field-gradient heteronuclear multiple-bond correlation (PFG-HMBC) spectroscopy. Analyses of the ¹H and ¹³C NMR spectra with the aid of DEPT, ¹H-¹H COSY and PFG heteronuclear multiple-quantum coherence (PFG-HMQC) spectra, coupled with the difference NOE experiments under the respective irradiation at 1-H and 7-H, enabled us to elucidate all of the proton spin system in **1**. The connectivity between those spin systems and the final planar structure of **1** were determined by detailed analysis of its PFG-HMBC spectrum. Then, the relative stereochemistry at C₃ and C₁₈ in **1** could be determined as shown in Fig. 1 on the basis of NOE's observed between 4-H and 19-H. Studies on the relative stereochemistry at C₁₂ and the absolute configuration of **1** are being undertaken currently.

The molecule of **1** has a unique structural skeleton with a spiro ring system composed from a γ -lactam fused to a benzene ring and a pentacyclic enamine fused to a diketopiperazine moiety, which are derived from a tryptophan unit, a proline residue and an isoprenyl group. The tryptophan unit in **1** has been modified by dihydrogenation at C₂/C₃, oxidation at C₂ and dehydrogen-

Fig. 7. Effect of **1** on the 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 spirotryprostatin B (12.5 μ g/ml) (c).

ation at C₈/C₉ positions. Although naturally occurring spiro compounds belonging to the diketopiperazine series derived from tryptophan and proline residues are known^{6~8,10}), none has a spiro ring skeleton similar to that of **1**. Tryptoquivalines have a spiro ring system composed from two heteropentacyclic rings¹¹), but their spiro rings are quite different from those in **1**. The present result provides spirotryprostatin (**1**), a novel natural diketopiperazine derivative having an unique spiro ring skeleton, as a new inhibitor of the mammalian cell cycle.

Spirotryprostatin B (**1**) completely inhibited the cell cycle progression of tsFT210 cells in the G2/M phase at final concentrations over 12.5 μ g/ml as shown in Fig. 7. In addition, **1** also showed cytotoxic activity on the growth of human chronic myelogenous leukemia K562 cells and human promyelocytic leukemia HL-60 cells with the MIC values of 35 μ g/ml and 10 μ g/ml, respectively. Further studies on the biological activities of **1** and the mechanism of its action are being undertaken currently.

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

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