COMMUNICATIONS TO THE EDITOR

Rasfonin, a New Apoptosis Inducer in *ras*-dependent Cells from *Talaromyces* sp.

Sir:

The oncogenic *ras* functions as an apoptosis suppressor as well as a cell-growth stimulator[1]. Thus, selective apoptosis inducers in *ras*-dependent cells are expected to be new anticancer agents against cells expressing constitutively active mutant *ras*. Hematopoietic cell-lines are appropriate for constructing a model of *ras*-dependent cells, because viability and proliferation of hematopoietic cells are strictly dependent on cytokines such as interleukins (IL) and colony-stimulating factors (CSF). The pro-B cell-line Ba/F3 can proliferate only in the presence of IL-3, and more than 90% of cells bearing dexamethazone-inducible v-H-*ras* (Ba/F3-V12) completely survive in an IL-3-free medium containing dexamethazone (Dex). In the course of our screening for apoptosis inducers in *ras*-dependent Ba/F3-V12 cells, a new active compound, rasfonin (Fig. 1) was isolated from the fermented mycelium of *Talaromyces* sp. 3656-A1. We describe herein the fermentation, isolation, physico-chemical properties, structure elucidation and biological activity of rasfonin.

The producing organism was incubated at 27°C for 14 days in 500-ml Erlenmeyer flasks containing a solid medium (brown rice 9 g, yeast extract 18 mg, Na tartarate 9 mg, KH_2PO_4 9 mg and H_2O 27 ml). The acetone extract of the culture (7 flasks) was evaporated to an aqueous concentrate and then partitioned between EtOAc and H_2O. The organic layer was subjected to silica gel column chromatography with $CHCl_3$-MeOH (30 : 1). The active eluate was purified by HPLC using a Senshu-Pak PEGASIL ODS column with 70% MeOH. The active fraction was concentrated to dryness to give a colorless oil (3.2 mg) of rasfonin: $[\alpha]_D^{22}$ −170° (*c* 0.086, MeOH); UV λ_{max}^{MeOH} nm (ε) 202 (16,300), 270 (22,800); IR ν_{max} (KBr) cm^{-1} 3450, 1730; FAB-MS *m/z* 435.2754 $(M+H)^+$, 435.2748 (calcd. for $C_{25}H_{39}O_6$).

The molecular formula of rasfonin was determined as $C_{25}H_{38}O_6$ by high-resolution FAB-MS. The ^{13}C and ^1H NMR spectra of rasfonin exhibited 25 carbon and 36 proton signals (Table 1). A heteronuclear multiple-quantum coherency (HMQC)[4] experiment established ^1H-^{13}C one-bond connectivities. A COSY experiment revealed four spin networks to generate partial structures **A** to **D** (Fig. 2). The heteronuclear multiple-bond correlation (HMBC)[5] spectrum of rasfonin displayed ^1H-^{13}C long-range couplings from 15-H_3 to C-9, C-10 and C-11, and from 9′-H_3 to C-3′, C-4′ and C-5′, indicating the connections between partial structures **A** and **B** *via* C-10, and between **C** and **D** *via* C-4′ (Fig. 2). ^1H-^{13}C long-range correlations from 4-H, 2′-H and 3′-H to a carbonyl carbon (C-1′) established an ester linkage between partial structures **A** and **C**. The remaining ester carbonyl carbon (C-1) was attached to C-2 based on ^1H-^{13}C long-range couplings from 2-H and 3-H to C-1.

Deuterium-induced upfield shifts were observed at C-8′ and C-10′ in $CDCl_3$ containing separately H_2O and D_2O, thereby showing that hydroxyl groups were located on C-8′ and C-10′. Since the molecular formula of rasfonin required the presence of a cyclic structure, the remaining oxymethine (C-5) was connected to C-1 with an ester linkage to form a δ-lactone ring. The geometrical configurations were determined to be 2*Z*, 10*E*, 2′*E*, 4′*E* by coupling constants ($J_{2\sim3}$=9.5 Hz and $J_{2'\sim3'}$=16.5 Hz) and upfield chemical shifts for allylic methyl carbons ($\delta_{C\text{-}15}$ 15.5 and $\delta_{C\text{-}9'}$ 12.6). Thus, the planar structure of rasfonin was determined as shown in Fig. 1. Rasfonin is an unsaturated δ-lactone bearing an alkyl chain at C-5 and an acyloxy chain at C-4. Although several δ-lactones possessing side chains have been reported[6,7], these compounds are not structurally related to rasfonin. Therefore, rasfonin seems to be a novel polyketide containing a δ-lactone.

Rasfonin induced cell death in Ba/F3-V12 cells in an IL-

Fig. 1. Structure of rasfonin.

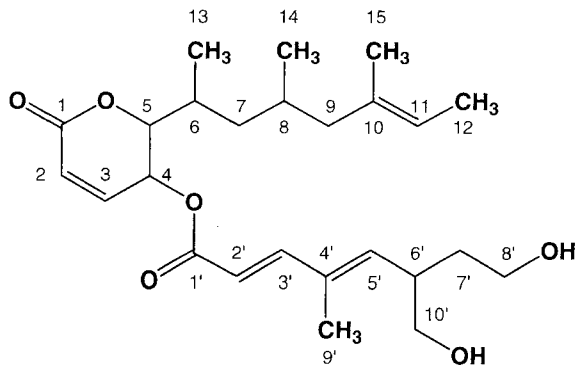
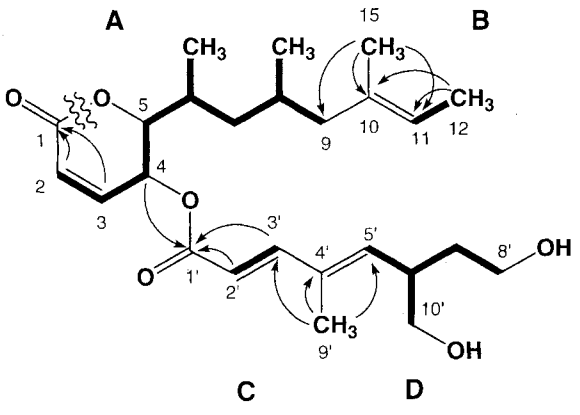


Table 1. ^{13}C (125 MHz) and ^{1}H NMR (500 MHz) data summary for rasfonin in $CDCl_3$.

No.	δ_C	δ_H (multiplicity, J = Hz)	No.	δ_C	δ_H (multiplicity, J = Hz)
1	163.3		14	20.6	0.76 (3H, d, 6.5)
2	124.9	6.19 (d, 9.5)	15	15.5	1.50 (3H, s)
3	140.6	7.02 (dd, 9.5, 6.0)	1'	166.1	
4	61.7	5.32 (dd, 6.0, 2.0)	2'	115.0	5.79 (d, 16.0)
5	83.3	4.11 (dd, 9.0, 2.0)	3'	150.9	7.32 (d, 16.0)
6	31.4	2.15 (m)	4'	134.7	
7	39.9	1.19 (ddd, 13.5, 9.0, 4.0)	5'	143.3	5.76 (d, 10.0)
		1.01 (ddd, 13.5, 10.0, 5.0)	6'	39.2	2.87 (m)
8	27.9	1.66 (m)	7'	34.7	1.78 (m)
9	46.3	2.04 (broad d, 13.0)			1.59 (m)
		1.42 (dd, 13.0, 10.0)	8'	60.6	3.72 (dt, 10.5, 5.0)
10	134.2				3.59 (m)
11	120.0	5.10 (q, 6.5)	9'	12.6	1.82 (3H, s)
12	13.3	1.53 (3H, d, 6.5)	10'	65.9	3.59 (m)
13	15.9	1.15 (3H, d, 6.5)			

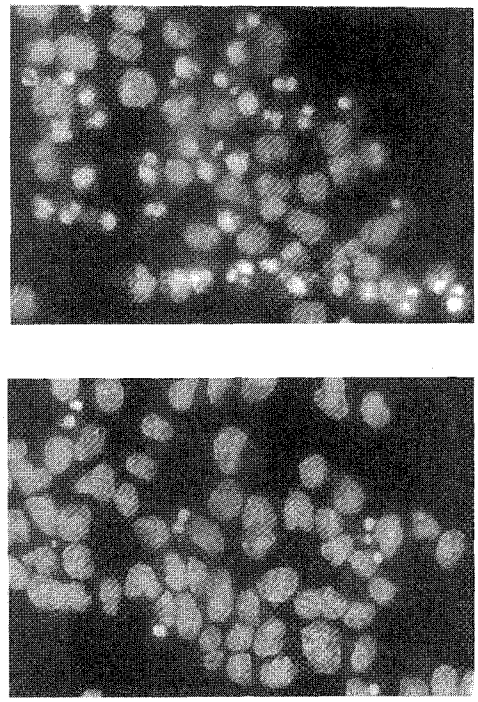
Fig. 2. Partial structures of rasfonin derived from COSY and HMBC experiments.

Bold lines show proton spin networks and arrows indicate ^{1}H-^{13}C long-range couplings.



3-free medium containing Dex (2×10^{-7} M) with an IC_{50} of 0.16 μg/ml. No cell death was observed in the presence of IL-3 at concentrations less than 1.25 μg/ml of rasfonin (IC_{50} 1.8 μg/ml). Significant numbers of *ras*-dependent Ba/F3-V12 cells treated with 1 μg/ml of rasfonin for 18 hours contained condensed chromatin and fragmented

Fig. 3. Fluorescence micrographs of Ba/F3-V12 cells stained with Hoechst Dye 33258.



Cells were cultured in the presence of Dex (2×10^{-7} M) for 18 hours after IL-3 withdrawal with (top) or without (bottom) 1 μg/ml of rasfonin.

nuclei as visualized by staining with Hoechst Dye 33258 (Fig. 3). The extract of these cells contained fragmented DNA (data not shown). These data suggest that the cell death induced by rasfonin resulted from apoptosis. Further studies on the biological activities of rasfonin are in progress.

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TAIJIRO TOMIKAWA
KAZUO SHIN-YA
KEIKO FURIHATA
TAISEI KINOSHITA
ATSUSHI MIYAJIMA
HARUO SETO
YOICHI HAYAKAWA*

Institute of Molecular and Cellular Biosciences,
The University of Tokyo,
Bunkyo-ku, Tokyo 113-0032, Japan

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