Selective Cytotoxicity and Stereochemistry of Aspochalasin D

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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*. In the course of our screening[2] for microbial products with selective cytotoxicity against *ras*-dependent Ba/F3 cells, *Aspergillus* sp. AJ117509 was found to produce an active substance (**1**).

The producing organism was cultivated in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of soluble starch 3.0%, sucrose 1.0%, Pharmamedia 1.0%, Polypepton 0.5% Distiller's soluble 0.5%, $MgSO_4$ 0.05%, $CaCO_3$ 0.3% and NaCl 0.1% (pH 6.0) on a rotary shaker at 25 °C for 6 days. The butanol extract of the culture (2 liters) 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 (20 : 1). The active eluate was purified by HPLC using a Senshu-Pak PEGASIL ODS column with 65% MeOH. The active fraction was concentrated to dryness to give a colorless powder (120 mg) of **1**.

The molecular formula of **1** was established to be $C_{24}H_{35}NO_4$ by high-resolution FAB-MS. The ^{13}C and ^1H NMR data of **1** are summarized in Table 1. The planar structure of **1** was elucidated by COSY, heteronuclear multiple-quantum coherency (HMQC)[3] and heteronuclear multiple-bond correlation (HMBC)[4] experiments (Fig. 1). Treatment of **1** with acetic anhydride in pyridine yielded the diacetyl derivative of **1**, which revealed ^1H and ^{13}C NMR spectra identical with those of aspochalasin D diacetate[5] (data not shown), thereby identifying **1** as aspochalasin D. In the previous report[5], the relative stereochemistry of aspochalasin D except for the diol moiety was established by comparison of the NMR data with those of its diastereomer, aspochalasin C (**3**), whose stereochemistry was determined by X-ray analysis[6]. The diol configurations were tentatively assigned as the 18-epimer of aspochalasin

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR assignments for aspochalasin D in $CDCl_3$.

No.	δ_C	δ_H (multiplicity, J = Hz)	No.	δ_C	δ_H (multiplicity, J = Hz)
1	175.1		14	137.3	
2		6.58 (s)	15	39.5	2.15 (2H, m)
3	51.2	3.12 (ddd, 9.5, 3.0, 3.0)	16	29.2	1.45 (m)
4	49.5	2.97 (dd, 6.0, 3.0)			2.05 (m)
5	35.0	2.45 (dq, 6.0, 6.0)	17	79.2	3.73 (broad s)
6	140.3		18	75.6	4.55 (m)
7	125.7	5.38 (broad s)	19	142.1	6.36 (dd, 16.5, 5.0)
8	43.6	2.88 (broad d, 10.5)	20	129.4	7.10 (d, 16.5)
9	68.2		21	197.6	
10	48.3	1.17 (m)	22	24.9	1.52 (m)
11	13.5	1.20 (3H, d, 6.0)	23	21.4	0.87 (3H, d, 7.0)
12	19.9	1.71 (3H, broad s)	24	23.5	0.85 (3H, d, 7.0)
13	124.1	5.90 (broad d, 10.5)	25	15.5	1.27 (3H, d, 0.5)

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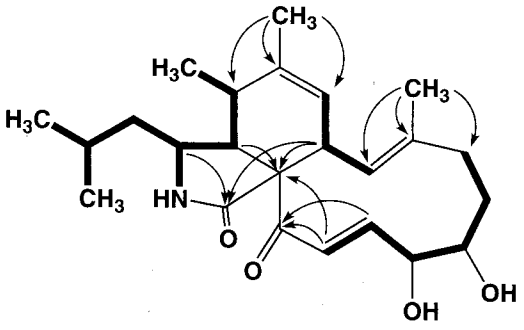
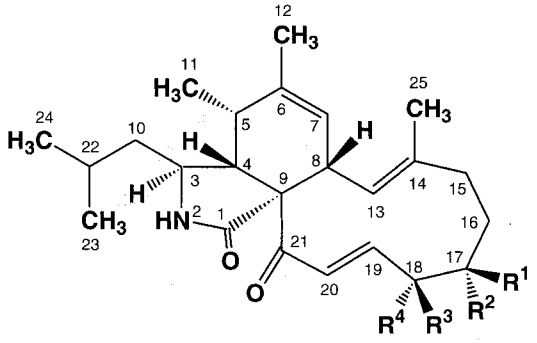
Fig. 1. COSY and HMBC data summary for **1**.



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

Fig. 2. Structures of aspochalasins D (**1**) and C (**3**).



1: R^1= OH, R^2= H, R^3= OH, R^4= H
2: R^1= H, R^2= OH, R^3= H, R^4= OH
3: R^1= H, R^2= OH, R^3= OH, R^4= H

C (**2**) based on the proton coupling constants (Fig. 2). To establish the configurations, we analyzed the stereochemical relationships of the protons in **1** by a NOESY experiment (Fig. 3). NOEs from H-8 to H-4 and H-5 indicated all β configurations for these protons. An NOE between H-3 and H-11 revealed α configurations for H-3 and 5-CH_3. H-25 displayed NOEs to H-8 and H-19, indicating that H-8, H-19 and 14-CH_3 existed on the β side of the 11-membered ring. On the other hand, NOEs from H-20 to H-13, H-17 and H-18, and from H-13 to H-17 required these protons to be on the α side of the ring. The *cis* configurations for the diol was also supported by an NOE and a small coupling constant between H-17 and H-18. These results identified **1** as the 17-epimer of

Fig. 3. Relative stereochemistry of **1** revealed by a NOESY experiment.

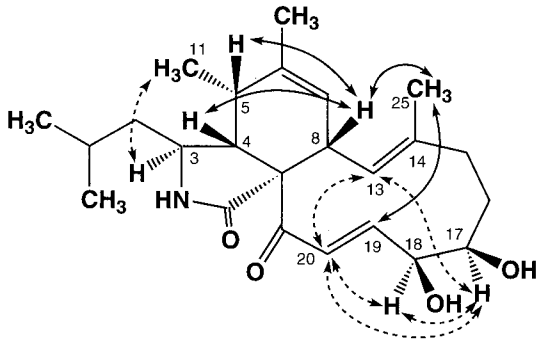
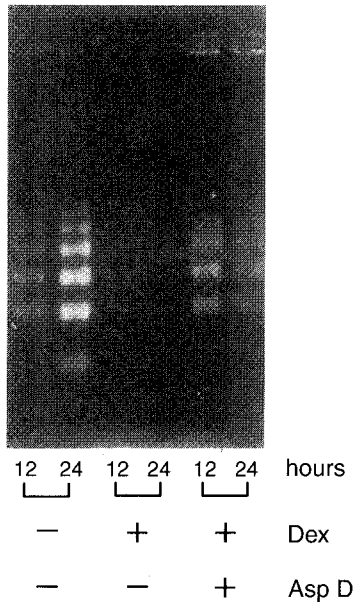


Fig. 4. Ethidium bromide-stained DNA extracted from Ba/F3-V12 cells.



Dex : dexamethasone (2×10^{-7} M),
Asp D : aspochalasin D (1 μg/ml).

aspochalasin C and then revised the previous stereochemical assignment.

More than 90% of IL-3-dependent Ba/F3 cells died within 24 hours after IL-3 withdrawal. However, Ba/F3 cells bearing oncogenic H-*ras* which is inducible by dexamethasone (Ba/F3-V12 cells) completely survived in an IL-3-free medium containing dexamethasone[7,8]. Aspochalasin D (**1**) induced cell death in Ba/F3-V12 cells

in an IL-3-free medium containing dexamethasone (2×10^{-7} M) with an IC_{50} of 0.49 μg/ml. No cell death was observed in the presence of IL-3 at concentrations less than 1.25 μg/ml of **1** (IC_{50} 1.9 μg/ml). The extract of *ras*-dependent Ba/F3-V12 cells treated with 1 μg/ml of aspochalasin D for 18 hours contained fragmented DNA (Fig. 4). However, only a small population of these cells contained condensed chromatin or fragmented nuclei as visualized by staining with Hoechst Dye 33258 (data not shown). These data suggested that **1** partially induced apoptotic signals in *ras*-dependent cells, whereas typical apoptosis was observed by IL-3 deprivation in Ba/F3 cells. Further studies on the biological activities of **1** are in progress.

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