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# Structure of Aspochalasin H, a New Member of the Aspochalasin Family

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We previously reported that aspochalasin D[1] showed selective cytotoxicity against *ras*-dependent Ba/F3 cells[2]. In this paper, we describe the structure of aspochalasin H (Fig. 1), a new member of the aspochalasin family produced by the aspochalasin D-producing strain, *Aspergillus* sp. AJ117509.

The fermentation was carried out 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 eluate was purified by HPLC using a Senshu-Pak PEGASIL ODS column with 65% MeOH. The purified fraction was concentrated to dryness to give a colorless powder (4.8 mg) of aspochalasin H.

The molecular formula of aspochalasin H was established to be $C_{24}H_{35}NO_5$ by high-resolution FAB-MS. The $^{13}C$ and $^1H$ NMR spectra of aspochalasin H exhibited 24 carbon and 35 proton signals (Table 1). A heteronuclear multiple-quantum coherency (HMQC)[3] experiment established $^1H$-$^{13}C$ one-bond connectivities as shown in Table 1. A COSY experiment revealed three spin networks to generate partial structures **A** to **C** (Fig. 2). Proton and carbon chemical shifts for 19- and 20-positions and their vicinal coupling constant (1.5 Hz) assigned this part to a *trans* epoxide moiety. The heteronuclaer multiple-bond correlation (HMBC)[4] spectrum of aspochalasin H displayed $^1H$-$^{13}C$ long-range couplings from 12-$H_3$ to C-5, C-6 and C-7, and from 25-$H_3$ to C-13, C-14 and C-15, indicating the connections between partial structures **A** and **B** *via* C-6, and between **B** and **C** *via* C-14 as shown in Fig. 2. A ketone carbonyl (C-21) revealed long-range couplings from 19-H and 20-H, and was required to be joined to C-20. $^1H$-$^{13}C$ long-range correlations from 4-H to an amide carbonyl (C-1) and a quaternary carbon (C-9) showed the presence of a $\gamma$-lactam ring. In the remaining connectivities, each C-8, C-9 and C-21 required additional carbon-carbon bonds from their chemical shifts, indicating that C-8 and C-21 were connected with C-9. The geometrical configuration of C-13 was determined to be *E* based on an upfield chemical shift for C-25 and an NOE between 8-H and 25-$H_3$. A NOESY experiment exhibited NOEs between 5-H and 8-H, between 4-H and 10-H, and between 3-H and 11-H, indicating $\beta$ configurations for 4-H, 5-H and 8-H, and an $\alpha$ configuration for 3-H (Fig. 3). An

Fig. 1. Structures of aspochalasins D and H.

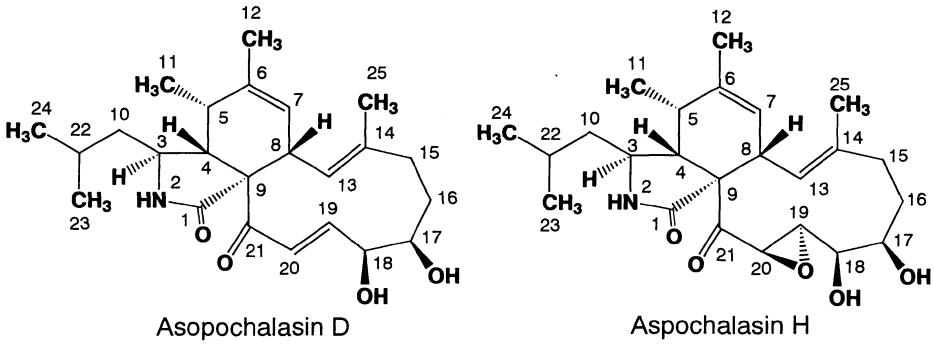


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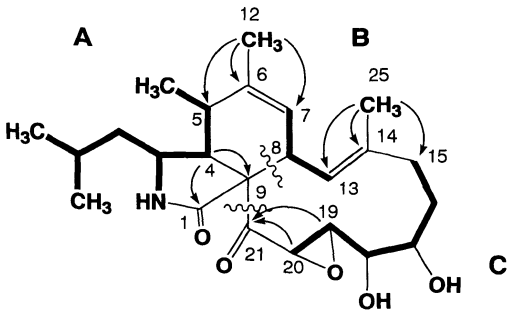
Table 1. $^{13}C$ (125 MHz) and $^{1}H$ (500 MHz) NMR assignments for aspochalasin H in $CDCl_3$.

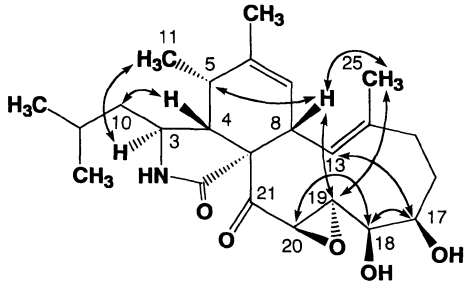
| No. | $\delta_C$ | $\delta_H$ (multiplicity, $J$ = Hz) | No. | $\delta_C$ | $\delta_H$ (multiplicity, $J$ = Hz) |
|---|---|---|---|---|---|
| 1 | 174.6 | | 14 | 136.0 | |
| 2 | | 6.06 (broad s) | 15 | 38.9 | 2.25 (t, 13.0) |
| 3 | 51.7 | 3.08 (dt, 10.0, 3.5) | | | 2.11 (m) |
| 4 | 52.8 | 2.64 (d, 3.5) | 16 | 29.3 | 2.12 (m) |
| 5 | 35.4 | 2.58 (m) | | | 1.53 (m) |
| 6 | 140.7 | | 17 | 72.9 | 4.04 (s) |
| 7 | 125.5 | 5.38 (m) | 18 | 78.4 | 3.37 (d, 7.0) |
| 8 | 44.1 | 3.02 (d, 11.0) | 19 | 61.7 | 2.62 (dd, 7.0, 1.5) |
| 9 | 67.4 | | 20 | 54.7 | 4.19 (d, 1.5) |
| 10 | 48.6 | 1.29 (m) | 21 | 205.6 | |
| | | 1.19 (m) | 22 | 25.2 | 1.53 (m) |
| 11 | 13.7 | 1.19 (3H, d, 7.0) | 23 | 23.6 | 0.90 (3H, d, 6.0) |
| 12 | 20.1 | 1.75 (3H, s) | 24 | 21.2 | 0.88 (3H, d, 6.0) |
| 13 | 124.6 | 5.98 (d, 11.0) | 25 | 15.5 | 1.42 (3H, s) |

Fig. 2. COSY and HMBC data summary for aspochalasin H.




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

Fig. 3. Relative stereochemistry of aspochalasin H revealed by a NOESY experiment.




NOE between 8-H and 19-H showed that the ketone carbonyl (C-21) was oriented to the $\beta$ side. NOEs between 8-H, 19-H and 25-$H_3$ required these protons to be on the $\beta$ side of the 11-membered ring. On the other hand, NOEs from 18-H to 17-H and 20-H, and from 13-H to 17-H revealed their $\alpha$ orientations. The *cis* configuration for the diol moiety was supported by a small coupling constant (<1 Hz) between 17-H and 18-H. The relative stereochemistry of aspochalasin H thus obtained was identical with that of aspochalasin D[2].

Aspochalasin H did not show cytotoxity against *ras*-dependent Ba/F3 cells at less than 25 $\mu$g/ml, whereas aspochalasin D induced cell death with an $IC_{50}$ of 0.49 $\mu$g/ml. The carbonyl-conjugated olefin at C-19 appeared to be significant for cytotoxicity of the aspochalasins.

Acknowledgment

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