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Aspergillin PZ, a Novel Isoindole-alkaloid from *Aspergillus awamori*

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Aspergillin PZ was obtained from the fermentation of *Aspergillus awamori* (Nakazawa) by activity-guided fractionation and purification. Its structure was elucidated on the basis of spectral data, especially by 2D NMR, and finally confirmed by an X-ray analysis. It could induce conidia of *P. oryzae* to deform moderately.

In the course of screening anti-tumor and anti-fungal agents from secondary metabolites of soil fungi using *Pyricularia oryzae* as the primary screening model, we found that the fermentation of *Aspergillus awamori* (Nakazawa) could induce mycelia of *P. oryzae* to geminate from conidia[1]. Aspergillin PZ was obtained from the fermentation broth by activity-guided fractionation, which posses a new carbon skeleton. Its structure was elucidated on the basis of spectral data, especially by 2D NMR, and finally confirmed by an X-ray analysis. In this paper, the production, isolation, biological activity, and structural elucidation are reported.

The fungus *A. awamori* was isolated from a soil sample collected from Heibei province, China. It was cultured using Sabourad's medium (Polypeptone (10 g/liter), NaCl (5 g/liter), Glucose (40 g/liter)) at 28°C and 260 rpm for 120 hours. The fermentation (7.0 liters) was partitioned with ethyl acetate.

The EtOAc extract (15 g) was chromatographed on a silica gel column (300 g, 8 cm×40 cm) with a solvent system of $CHCl_3$ - MeOH (100 : 2) and further recrystalized in $CHCl_3$ - MeOH (1 : 10), to give aspergillin PZ (100 mg).

Aspergillin PZ was obtained as colorless square crystal. Its molecular formula was inferred by HREI-MS (*m/z* 401.2571) as $C_{24}H_{35}NO_4$. The ^{13}C NMR and DEPT of it displayed five methyls signals (δ 13.4, 19.9, 21.4, 23.3, 23.9), four methylenes (δ 24.7, 34.7, 42.6, 48.1), nine methines (δ 24.0, 34.3, 35.8, 38.7, 42.4, 51.1, 51.6, 65.2, 83.4), 2 quaternary carbons (δ 63.9, 81.4), 2 olefinic carbons (δ 127.5, 138.7) and two carbonyl carbons (δ 173.1, 211.6). Among these signals, δ 127.5 (C-6) and 138.7 (C-5) indicated the presence of a trisubstituted double bond group, δ 211.6 and 173.1 suggested the presences of a carbonyl group and an amide carbonyl group, respectively.

In the ^1H-^1H COSY spectrum, the correlations of δ 1.70 (H-2′) to δ 0.86 (2′-CH_3, H_3-3′) and 1.20 (H_A-1′); δ 2.90 (H-3) to δ 1.20 (H_A-1′), 1.48 (H_B-1′), 2.38 (H-3a) and 8.00 (2-NH); δ 2.47 (H-4) to δ 1.07 (4-CH_3) and 2.38 (H-3a) indicated the fragment A as shown (Fig. 1). The moiety B was deduced by correlations of δ 1.51 (H_A-9) to δ 1.32 (H_A-8), 1.79 (H_B-9), 1.81 (H_B-8) and 3.38 (H-10); δ 3.38 (H-10) to δ 3.50 (H-11), 4.49 (OH). Furthermore, the correlations of δ 2.67 (H-11a) to δ 2.29 (H_A-12), 2.56 (H_B-12) and 2.77 (H-6b); δ 2.50 (H-6a) to δ 2.77 (H-6b) and 5.44 (H-6); δ 5.44 (H-6) to 1.72 (5-CH_3) suggested the presence of fragment C (Fig. 1).

The long range ^1H-^{13}C correlations of δ 1.10 (3H, s, 7α-CH_3) to δ 81.4 (C-7), 34.7 (C-8) and 42.4 (C-6b); δ 3.50 (H-11) to δ 42.6 (C-12); δ (2.29 and 2.56, each 1H, m, H-12) to δ 211.6; δ 1.07 (4β-CH_3) to δ 138.7 (C-5); δ 8.00 (2-NH) to δ 173.1 (C-1) and 63.9 (C-13a) in HMBC spectrum (Fig. 2) led to determination of the connectivities of three fragments (A, B and C, Fig. 1) in the molecule. All protons and carbons were assigned on the basis of ^1H-^1H COSY, HMQC and HMBC spectra (Table 1). Aspergillin PZ was designated as 3β, 3aα, 4α, 6aα-tetrahydro-10α-hydroxy-4β,5,7α-trimethyl-3α-(2-methylpropyl)-[*cis*-transoid-*trans*-(12-oxatricyclo[6.3.1.$0^{2,7}$])]dodeca[4,3-d]isoindole-1 (*H*), 13-dione. The structure was finally verified by an X-ray analysis (Fig. 3). The skeletal structure

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Fig. 1. Structure fragments of aspergillin PZ, and ^{1}H-^{1}H COSY of it.

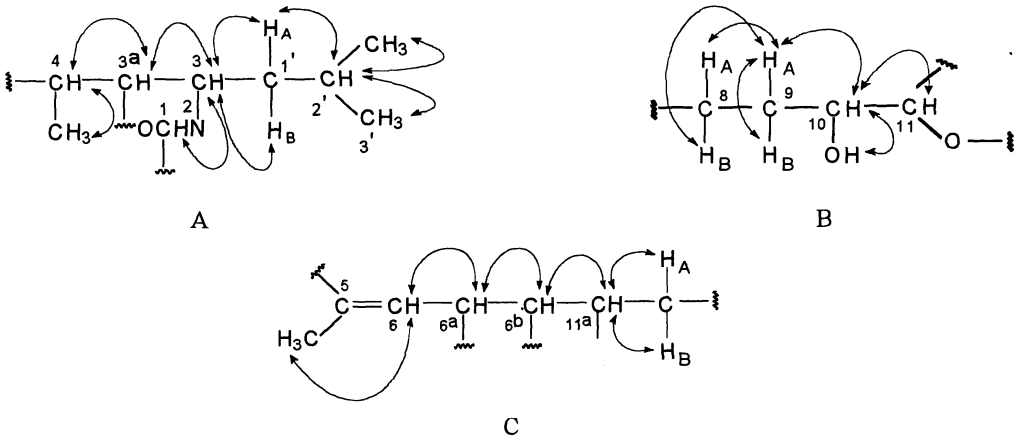

Fig. 2. Key HMBC correlations of asepergillin PZ.

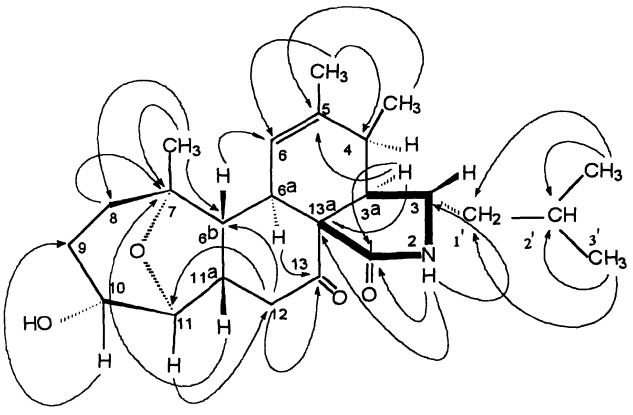

Fig. 3. ORTEP drawing of aspergillin PZ.

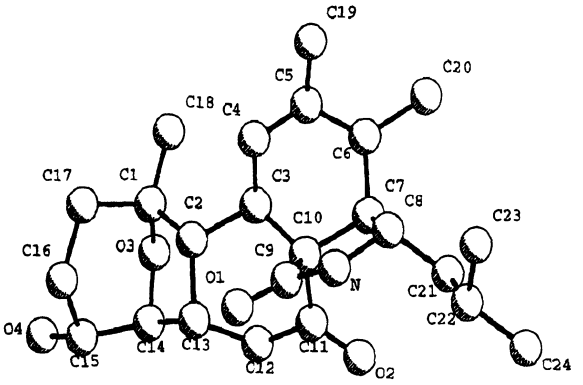

Table 1. NMR data of aspergillin PZ.

No.	^{1}H-NMR	^{13}C-NMR	No.	^{1}H-NMR	^{13}C-NMR
1		173.1	**11**	3.50 (1H, s)	83.4
2	8.00 (1H, s)		**11a**	2.67 (1H, m)	38.7
3	2.91 (1H, m)	51.1	**12**	2.29 (1H, m, H_A), 2.56 (1H, m, H_B)	42.6
3a	2.38 (1H, m)	51.6			
4	2.47 (1H, m)	34.3	**13**		211.6
5		138.7	**13a**		63.9
6	5.44 (1H, s)	127.5	**1'**	1.20 (1H, m, H_A), 1.48 (1H, m, H_B)	48.1
6a	2.50 (1H, m)	35.8			
6b	2.77 (1H, m)	42.4	**2'**	1.70 (1H, m)	24.0
7		81.4	**2'-Me**	0.86 (3H, t, J =6.6Hz)	21.4*
8	1.32 (1H, m, H_A), 1.81 (1H, m, H_B)	34.7	**3'**	0.86 (3H, t, J =6.6Hz)	23.9*
			4β-Me	1.07 (3H, d, J =7.2Hz)	13.4
9	1.51 (1H, m, H_A), 1.79 (1H, m, H_B)	24.7	**5-Me**	1.72 (3H, s)	19.9
			7α-Me	1.10 (3H, s)	23.3
10	3.38 (1H, m)	65.2	**10α-OH**	4.49 (1H, d, J =4.8Hz)	

^{1}H NMR (300 MHz, DMSO-d_6), ^{13}C NMR (75 MHz, DMSO-d_6)

*: data of them can be exchanged.

of aspochalasin C was reported to be very similar to aspergillin PZ[2]. The addition of 18-OH of the former compound followed by cyclization would give the latter one. The relative stereochemistry of C-3, C-3a, C-4 and C-13a of aspergillin PZ was related to the counterpart of aspochalasin C.

In previous research, we found that the analogs of aspergillin PZ isolated from *aspergillum* genus could induce conidia of *P. oryzae* to deform moderately. Furthermore, they had activity against cancer cells such as MH-60 and HL-60, which indicated the connection between anti-tumor and anti-*P. oryzae*[3] activities. Pharmacological research indicated aspergillin PZ could induce morphological deformation of conidia of *P. oryzae* strongly at 0.089 μM. This evidence suggested that the compound had activity to tumor cell. Moreover, the content of it in EtOAc extracts was 0.833%, which was very high. So we can obtain it by improving the fungus if it has activities to cancer cells. Further pharmacological research of it is now under way in our laboratory.

Acknowledgments

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