ASPOCHALASIN E, A NEW ANTIBIOTIC ISOLATED FROM A FUNGUS

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In the course of screening for new biological actives, an unidentified fungal strain (FA2277) was found to produce several cytotoxic substances including a new aspochalasin analog. Aspochalasins which are structurally related to cytochalasins, well-known mycotoxins, were first isolated as metabolic products of *Aspergillus microcysticus*[1], and few compounds of this group have been characterized to date[2,3]. This paper briefly describes the fermentation, isolation, structural studies, and cytotoxic activity of aspochalasin E, a new aspochalasin analog (Fig. 1).

Strain FA2277 was isolated from a soil sample collected in Kawasaki City, Kanagawa Prefecture, Japan. A loopful spores of this strain was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of vegetative medium composed of soluble starch 2%, soybean meal 1% and $CaCO_3$ 0.5%, pH 7.0 before sterilization, and incubated at 28°C for 4 days on a rotary shaker (200 rpm). For production of

Scheme 1. Purification procedure of seven components.

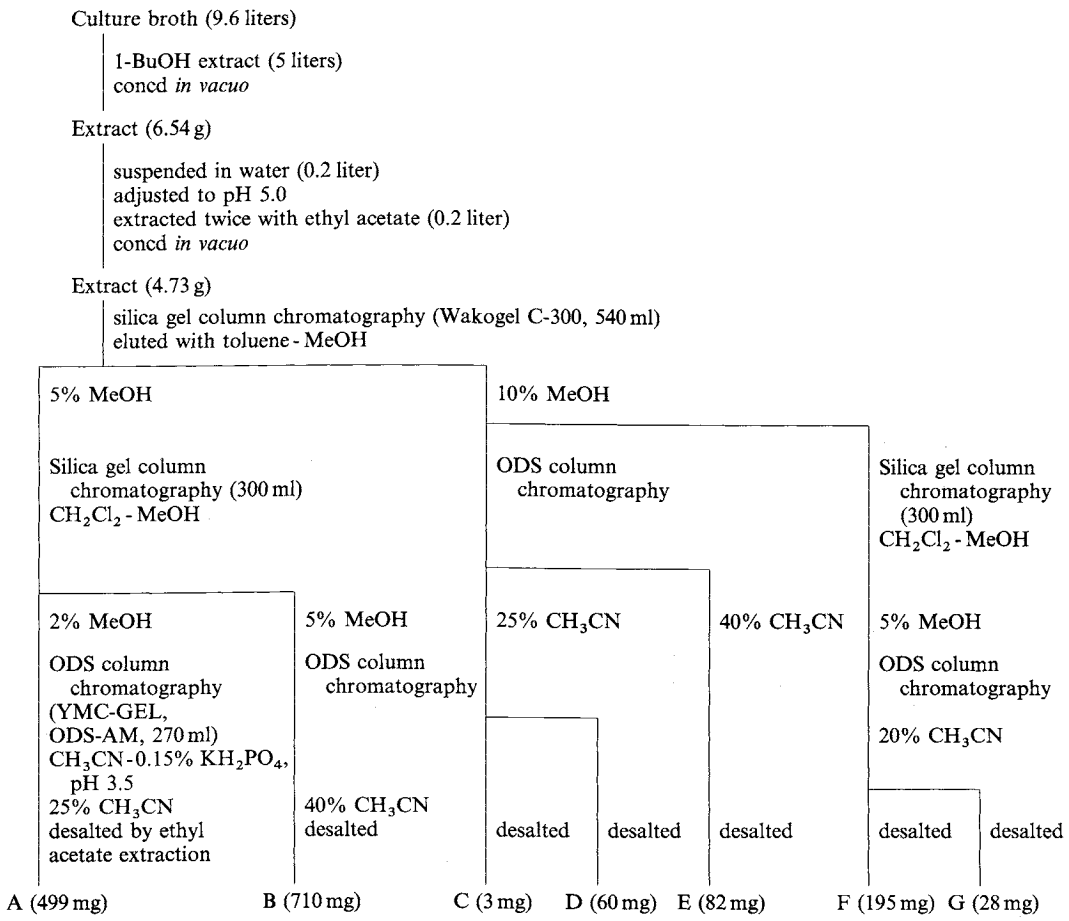

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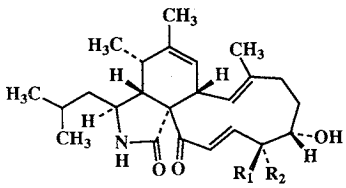
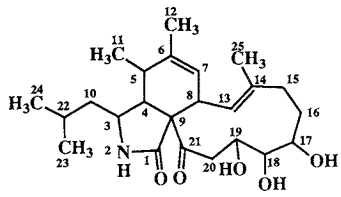
Fig. 1. Structures of aspochalasins C (1), D and E (2).



Aspochalasin C: $R_1=OH$
(Component B, 1) $R_2=H$
Aspochalasin D: $R_1=H$
$R_2=OH$



Aspochalasin E (2)

Table 1. Physico-chemical properties of aspochalasin E (2).

Nature	White powder
MP	129~131°C
$[\alpha]_D^{24}$	−52° (*c* 0.5, $CHCl_3$)
Formula	$C_{24}H_{37}NO_5$
FAB-MS ($(M+H)^+$, *m/z*)	
Calcd:	420.2750
Found:	420.2749
UV λ_{max}^{MeOH} nm	End absorption
IR ν_{max}^{KBr} cm^{-1}	3400, 2900, 1690, 1445, 1385, 1065

Table 2. ^{13}C and ^1H NMR data for aspochalasin E (2) in DMSO-d_6.

No.	δ_C (in ppm)	δ_H (in ppm)
1	174.4	
2		8.12 s
3	49.8	3.07 m
4	51.7	2.41 m
5	34.9	2.42 m
6	139.0	
7	125.4	5.33 m
8	43.0	3.05 m
9	67.2	
10	48.7	1.04 m
11	13.0	1.16 d, 6.8 Hz
12	19.4	1.72 br s
13	124.1	6.02 d, 10.7 Hz
14	135.7	
15	38.0	1.96 m
16	29.4	1.30 m,
		1.54 m
17	70.5	3.60 m
18	78.4	3.25 m
19	67.7	3.17 m
20	43.8	1.76 dd, 17.5, 5.1 Hz
		3.90 d, 17.5 Hz
21	210.9	
22	23.9	1.57 m
23	23.4	0.834 d, 6.4 Hz
24	21.7	0.831 d, 6.4 Hz
25	15.5	1.38 d, 0.9 Hz
17-OH		4.43 d, 6.0Hz
18-OH		4.37 m
19-OH		4.38 d, 4.3 Hz

antibiotics 5 ml of the culture was transferred into 500-ml Erlenmeyer flasks containing 100 ml each of fermentation medium having the same composition as the vegetative medium. The fermentation was carried out at 28°C for 5 days on a rotary shaker.

The isolation procedure of cytotoxic components from culture broth of strain FA2277 is schematically shown in Scheme 1. From the 1-butanol extract of the fermentation broth (9.6 liters), components A (499 mg), B (710 mg), C (3 mg), D (60 mg), E (82 mg), F (195 mg) and G (28 mg) were isolated as homogeneous powders.

Components A, C, D, F and G were all revealed to be curvularin-type metabolites and were spectroscopically identified as known α,β-dehydrocurvularin (component A)[4] and the stereoisomers of β-methoxycurvularin (C, D)[5,6] and β-hydroxycurvularin (F, G)[7,8]. On the other hand, both components B (1) and E (2) contained nitrogen atoms in their molecules and were shown to belong to the aspochalasin group of antibiotics. 1 was deduced to be aspochalasin C[3] by spectroscopic analyses.

The physico-chemical properties of component E (2) are summarized in Table 1. The molecular formula of 2, $C_{24}H_{37}NO_5$, determined by HRFAB-MS indicates that 2 is a hydroxylated compound of aspochalasin C or D. The ^{13}C and ^1H NMR data of 2 are presented in Table 2. All one-bond ^1H-^{13}C correlations were established by ^1H-^{13}C COSY spectrometry, indicating the presence of two separate spin systems as shown in Fig. 2. The long-range ^1H-^{13}C COSY spectrum (12 Hz, Fig. 2) allows to

Fig. 2. Proton spin systems derived from a COSY experiment and the long-range ^{1}H-^{13}C COSY data for aspochalasin E (**2**).

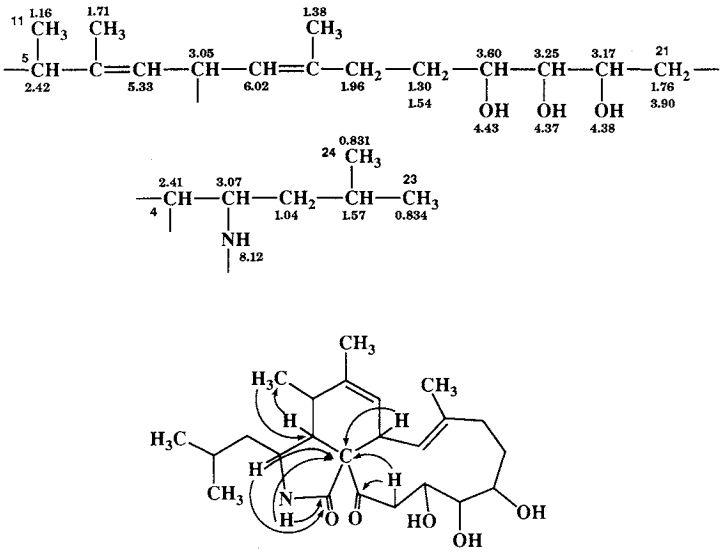


Table 3. *In vitro* cytotoxicity.

Compound	IC_{50} (μg/ml)	
	B16-F10	HCT-116
Aspochalasin C	3.2	1.5
Aspochalasin E	18.5	6.3

connect these two partial structures, leading to the planar structure of **2** as a 19-hydroxy analog of aspochalasin C or D (Fig. 1). Although the stereochemical studies of **2** has not yet been performed, **2** is assumed to have the same stereochemistry as co-produced aspochalasin C (**1**) or D. Accordingly the name of aspochalasin E is proposed for **2**.

Seven components all inhibited the growth of murine melanoma B16-F10 and human colon carcinoma HCT-116 cells. The IC_{50} values of aspochalasin E (**2**) and component B (**1**) are listed in Table 3.

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