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Structure Elucidation of Fungal Phenochalasins, Novel Inhibitors of Lipid Droplet Formation in Mouse Macrophages

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The structures of phenochalasins A and B were elucidated by spectroscopic studies including various NMR measurements. Phenochalasins A and B have the cytochalasan skeleton of the 21,23-dioxa, 17,22-dione moiety containing unique phenyl and *O*-methyl phenyl residues at the C-10 position, respectively.

Phenochalasins A and B (Fig. 1) were isolated as inhibitors of lipid droplet formation in mouse peritoneal macrophages from the culture broth of *Phomopsis* sp. FT-0211. The fermentation, isolation and their biological properties are described in the preceding paper[1]. We report herein the structure elucidation of phenochalasins.

Materials and Methods

Chemicals

Cytochalasin E was purchased from Sigma.

General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Optical rotations were obtained with a JASCO DIP-370 digital polarimeter. Melting points were measured with a Yanaco micro melting point apparatus. EI-MS spectra were recorded on a JEOL JMS-D 100 mass spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer.

Results

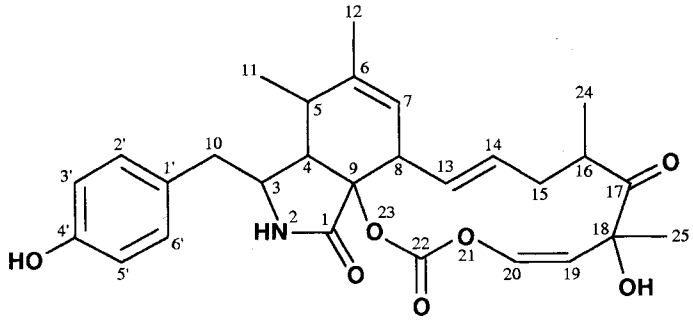
Physico-chemical Properties of Phenochalasins

Physico-chemical properties of phenochalasins are summarized in Table 1. They showed the similar UV absorption maxima at 200, 225 and 275~300 nm. The IR absorption at 3403 and 1749~1709 cm^{-1} suggested the presence of hydroxy and carbonyl groups in the structures[2].

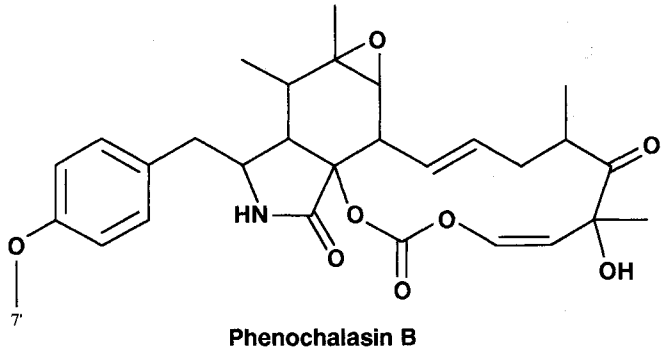
Structure of Phenochalasin A

The molecular formula of phenochalasin A was determined to be $C_{28}H_{33}NO_7$ on the basis of HRFAB-MS measurement. The ^{13}C NMR spectrum ($CDCl_3$) showed 26 resolved peaks corresponding to 28 carbons (Table 2), which were classified into four methyl, two methylene, four methine, one *N*-methine, nine sp^2 methine, two sp^3 quaternary, three sp^2 quaternary and three carbonyl carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 30 proton signals (Table 2). To fulfill the molecular formula of phenochalasin A, the presence of two hydroxyl groups and one amido group was suggested. The connectivity of proton and carbon atoms was established by the ^{13}C-^1H HMQC spectrum (Table 2). Analysis of the ^1H-^1H COSY spectrum revealed the four partial structures I to IV (Fig. 2). ^{13}C-^1H long range couplings of ^2J and ^3J observed in the ^{13}C-^1H HMBC experiment (Fig. 3) gave the following evidence: 1) The cross peaks from H-3 (δ 3.30)

Fig. 1. Structures of phenochalasins A and B and cytochalasin E.



Phenochalasin A



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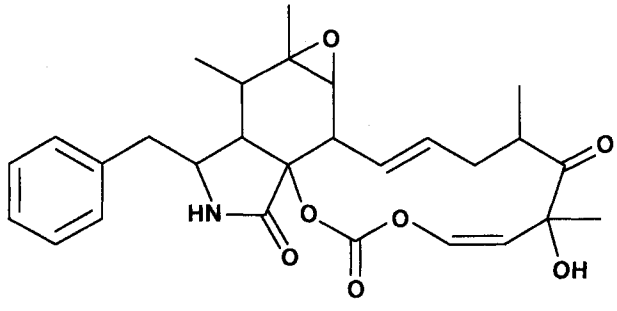


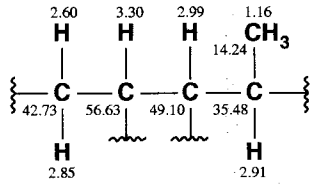
Cytochalasin E

to C-1′ (δ 128.21), from H_2-10 (δ 2.60, 2.85) to C-1′ and C-2′ (δ 132.33), from H-2′ (δ 6.96) to C-4′ (δ 157.45) and C-6′, and from H-3′ (δ 6.71) to C-1′, C-4′ and C-5′ (δ 116.20) showed the presence of a 4′-hydroxy benzyl moiety containing the partial structure IV. A fragment ion peak (*m/z* 107) of FAB-MS, ^{13}C NMR chemical shifts[2] and IR spectrum also supported the presence of 4′-hydroxyl benzyl group. 2) The cross peaks from H-4 (δ 2.99) to C-6 (δ 143.14) and C-9 (δ 91.72), from H-5 (δ 2.91) to C-6 and C-12 (δ 19.90), from H-7 (δ 5.23) to C-5 (δ 35.48) and C-9, from H-8 (δ 3.02) to C-7 (δ 123.79) and C-9, from H_3-11 (δ 1.16) to C-6, and from H_3-12 (δ 1.77) to C-5, C-6 and C-7 indicated a 4,5,6,8,9-pentasubstituted cyclohexene containing the partial structure I. 3) The long-range couplings from H-3, H-4 and H-8 to C-1 (δ 172.70) suggested that a pyrrole ring was attached to the cyclohexene ring. 4) The long-range couplings from H-8 to C-13 (δ 130.79), and from H-13 (δ 5.63) to C-7 and C-8 (δ 46.04) suggested that partial structure II was attached to the cyclopentene ring. The proton coupling constant was 15.0 Hz between H-13 and H-14 (δ 5.20), indicating that the olefin has the trans configuration. 5) The long-range couplings from H_2-15 (δ 2.10, 2.46) to C-17 (δ 213.35), from H-16 (δ 3.08) to C-17, from H-19 (δ 5.79) to C-17, C-18 (δ 78.75) and C-25 (δ 24.42), from H-20 (δ 6.38) to C-18, from H_3-24 (δ 1.10) to C-17, and from H_3-25 (δ 1.46) to C-17, C-18 and C-19 (δ 122.49) suggested the sequence of II-^{17}C=O−$^{18}C(^{25}CH_3)$−III. And ^{13}C chemical

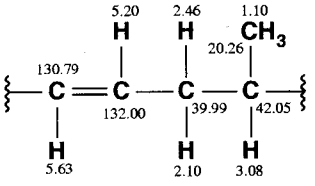
Table 1. Physico-chemical properties of phenochalasins A and B.

	Phenochalasin A	Phenochalasin B
Appearance	White powder	White powder
Molecular formula	$C_{28}H_{33}NO_7$	$C_{29}H_{35}NO_8$
Molecular weight	495	525
FAB-MS (*m/z*)		
Positive	496 $[M+H]^+$	526 $[M+H]^+$
	518 $[M+Na]^+$	548 $[M+Na]^+$
Negative	494 $[M-H]^-$	524 $[M-H]^-$
HRFAB-MS (*m/z*) (negative)		
	$C_{28}H_{33}NO_7$	$C_{29}H_{35}NO_8$
Calcd:	496.2335	526.2441
Found:	496.2344	526.2439
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	200 (27,800)	200 (24,500)
	225 (10,900)	225 (14,400)
	285 (3,000)	275 (3,200)
	300 (2,100)	283 (2,900)
IR ν_{max}^{KBr} (cm^{-1})	3403, 1749, 1709,	3403, 1767, 1718,
	1664, 1614, 1595,	1662, 1612, 1514,
	1442	1456, 1311
$[\alpha]_D^{22}$ (*c* 0.53, CH_3OH)	− 4.3 °	− 5.7 °
melting point	143~145°C	130~132 °C
Solubility		
Soluble:	CH_3OH, $CHCl_3$,	CH_3OH, $CHCl_3$,
	CH_3CN, Acetone,	CH_3CN, Acetone,
	C_2H_5OH, Ethyl acetate	C_2H_5OH, Ethyl acetate
Insoluble:	H_2O, *n*-Hexane	H_2O, *n*-Hexane
Color reaction		
Positive:	50% H_2SO_4	50% H_2SO_4

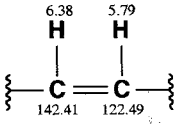
Fig. 2. Partial structures I to IV of phenochalasin A.



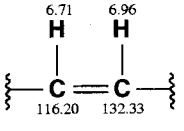
I



II



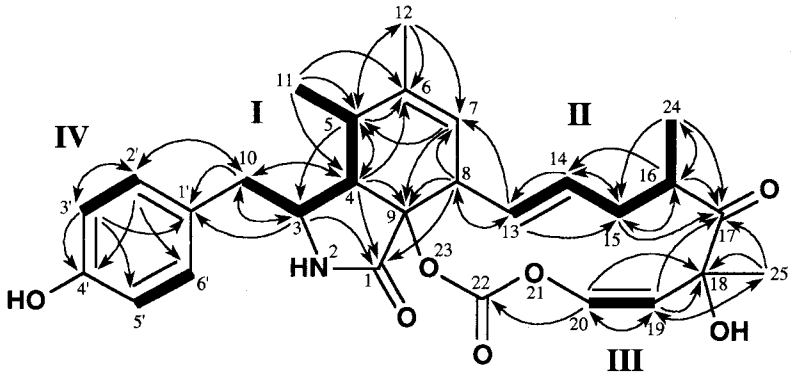
III



IV

shift[2] (δ 78.75) supported the presence of a hydroxyl group at C-18. The proton coupling constant was 12.0 Hz between H-19 and H-20, indicating that the olefin has the *cis* configuration. 6) The long-range coupling was observed from H-20 to C-22 (δ 151.19), and the chemical shift of δ 151.19 was comparable with that of a dioxa carbonyl carbon[3]. Finally cytochalasan skeleton was suggested because of the degree of unsaturation and the molecular formula. Taken together, the structure of phenochalasin A was elucidated as shown in Fig. 1.

Fig. 3. ^{1}H-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC experiments of phenochalasin A.



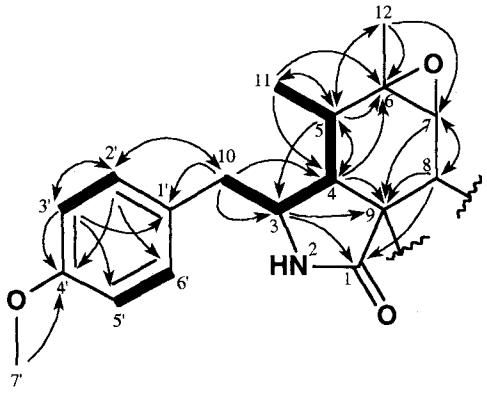
^{1}H-^{1}H COSY: ——, ^{13}C-^{1}H HMBC: H⟶C

Table 2. ^{1}H and ^{13}C NMR chemical shifts of phenochalasins A and B and cytochalasin E.

Carbon No.	Phenochalasin A		Phenochalasin B		Cytochalasin E
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]
C-1	172.70		169.81		168.9
C-3	56.63	3.30 (1H, ddd, *J*=5.5, 4.0, 4.0 Hz)	53.77	3.65 (1H, ddd, *J*=8.0, 4.0, 3.0 Hz)	52.1
C-4	49.10	2.99 (1H, dd, *J*=4.0, 4.0 Hz)	48.27	2.99 (1H, dd, *J*=5.0, 3.0 Hz)	45.6
C-5	35.48	2.91 (1H, m)	35.87	2.30 (1H, d, *J*=7.5, 5.0 Hz)	35.5
C-6	143.14		57.26		56.1
C-7	123.79	5.23 (1H, d, *J*=1.0 Hz)	60.61	2.63 (1H, s)	58.8
C-8	46.04	3.02 (1H, dm, *J*=10.0 Hz)	46.02	2.64 (1H, d, *J*=10.0 Hz)	45.9
C-9	91.72		86.99		86.9
C-10	42.73	2.60 (1H, dd, *J*=14.0, 5.5 Hz) 2.85 (1H, dd, *J*=14.0, 4.0 Hz)	44.31	2.59 (1H, dd, *J*=14.0, 8.0 Hz) 2.82 (1H, dd, *J*=14.0, 4.0 Hz)	42.6
C-11	14.24	1.16 (3H, d, *J*=7.5 Hz)	13.25	1.12 (3H, d, *J*=7.5 Hz	12.4
C-12	19.90	1.77 (3H, d, *J*=1.0 Hz)	19.69	1.25 (3H, s)	19.8
C-13	130.79	5.63 (1H, ddd, *J*=15.0, 10.0, 2.0 Hz)	128.38	5.89 (1H, ddd, *J*=15.0, 10.0, 1.5 Hz)	127.6
C-14	132.00	5.20 (1H, ddd, *J*=15.0, 10.5, 4.0 Hz)	131.64	5.23 (1H, ddd, *J*=15.0, 11.0, 4.0 Hz)	131.4
C-15	39.99	2.10 (1H, dm, *J*=14.0 Hz) 2.46 (1H, ddd, *J*=14.0, 11.0, 10.5 Hz)	39.07	2.14 (1H, dddd, *J*=14.0, 4.0, 2.5, 1.5 Hz) 2.65 (1H, ddd, *J*=14.0, 11.5, 11.0 Hz)	38.5
C-16	42.05	3.08 (1H, ddd, *J*=11.0, 7.0, 3.0 Hz)	40.85	2.94 (1H, ddd, *J*=11.5, 7.0, 2.5 Hz)	39.6
C-17	213.35		211.72		211.4
C-18	78.75		76.74		76.5
C-18-OH				4.43 (1H, s)	
C-19	122.49	5.79 (1H, d, *J*=12.0 Hz)	120.42	5.62 (1H, d, *J*=12.0 Hz)	121.0
C-20	142.41	6.38 (1H, d, *J*=12.0 Hz)	142.17	6.53 (1H, d, *J*=12.0 Hz)	140.5
C-22	151.19		149.36		148.8
C-24	20.26	1.10 (3H, d, *J*=7.0 Hz)	20.08	1.16 (3H, d, *J*=7.0 Hz)	19.1
C-25	24.42	1.46 (3H, s)	24.36	1.50 (3H, s)	24.8
C-1'	128.21		127.97		136.1
C-2', C-6'	132.33	6.96 (2H, dd, *J*=8.5, 2.0 Hz)	130.47	7.05 (2H, dd, *J*=8.5, 2.0 Hz)	130.1
C-3', C-5'	116.20	6.71 (2H, dd, *J*=8.5, 2.0 Hz)	114.39	6.86 (2H, dd, *J*=8.5, 2.0 Hz)	127.9
C-4'	157.45		158.92		126.3
C-7'			55.29	3.79 (3H, s)	

a) Chemical shifts are shown with reference to $CDCl_3$ as 77.7 ppm. b) Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.

Fig. 4. ^{1}H-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC experiments of phenochalasin B.



^{1}H-^{1}H COSY: ——, ^{13}C-^{1}H HMBC: H⟶C

Structure of Phenochalasin B

The molecular formula $C_{29}H_{35}NO_8$ of phenochalasin B was CH_2O bigger than phenochalasin A. The ^{13}C NMR spectrum of phenochalasin B (Table 2) was similar to that of phenochalasin A, but the three carbon signals of C-7′, C-6 and C-7 were different, that is, the methoxy C-7′ (δ 55.29), the oxyquaternary C-6 (δ 57.26) and the oxymethine C-7 (δ 60.61) carbons in phenochalasin B. The structural analyses were done by ^{1}H-^{1}H COSY and HMBC experiments as shown in Fig. 4. The long-range couplings from H_3-7′ (δ 3.79) to C-4′ (δ 158.92) suggested that a methoxy residue was attached to the benzyl moiety. The long-range couplings were observed from H-4 (δ 2.99) to C-6 and C-9 (δ 86.99), from H-5 (δ 2.30) to C-6 and C-12 (δ 19.69), from H-7 (δ 2.63) to C-8 (δ 46.02) and C-9, from H-8 (δ 2.64) to C-7 and C-9, from H_3-11 (δ 1.12) to C-6, and from H_3-12 (δ 1.25) to C-5 (δ 35.87), C-6 and C-7, indicating a 4,5,6,7,8,9-hexasubstituted cyclohexane ring. And the chemical shift of C-6 (δ 57.26) and C-7 (δ 60.61) carbons were comparable with those of epoxy carbons. Taken together, the structure of phenochalasin B was elucidated as shown in Fig. 1.

Discussion

Fungal phenochalasins A and B were isolated as novel inhibitors of lipid droplet formation in mouse macrophages[1]. In the present paper, their planar structures were elucidated mainly by NMR studies. They were found to belong to the cytochalasan family and were very similar to cytochalasin E[3], whose ^{13}C chemical shifts are added to Table 2 for comparative purpose. Consequently, phenochalasin B is 4′-methoxy cytochalasin E. About 40 cytochalasan derivatives of fungal origin have been reported[5]. The structural characteristics of phenochalasins A and B are the presence of phenyl and methyl phenyl residues at the C-10 positions instead of benzene, indole and isopropyl residues in known cytochalasans. Only pyrichalasin H was reported to have a methyl phenyl residue at the position[4]. As described in the preceding paper, phenochalasin A inhibits the lipid droplet formation without toxic effect on macrophages, while phenochalasin B showed very cytotoxic effect[1]. The presence of the epoxide at the C-6 and C-7 position might be responsible for eliciting cytotoxic activity.

Acknowledgment

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