STRUCTURE-ACTIVITY RELATIONSHIP OF THIRTY-NINE CYTOCHALASANS OBSERVED IN THE EFFECTS ON CELLULAR STRUCTURES AND CELLULAR EVENTS AND ON ACTIN POLYMERIZATION *IN VITRO*

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The effects of twenty-three natural cytochalasans and sixteen synthetic derivatives on actin-distribution and alteration in morphology of C3H-2K cells, inhibition of lymphocyte capping, and inhibition of actin filament elongation were compared. The effects on cellular level and the *in vitro* effects showed positive correlation and the structure-activity relationship observed is discussed.

Keywords — cytochalasan; cytochalasin; chaetoglobosin; lymphocyte capping; actin polymerization; structure-activity relationship

The cytochalasans, a group of fungal secondary metabolites, inhibit a variety of cellular movements, including cell division and motility, and cause change in cell shape.¹⁾ In a previous paper²⁾ the effects of twenty natural cytochalasans among forty so far known^{3,4)} and four acetates prepared were determined, both on the cellular level and on the molecular level, four high dose effects on the cellular level (rounding up of fibroblastic cells, contraction of actin cables, formation of hairy filament containing actin, and inhibition of lymphocyte capping), a low dose effect on the cellular level (inhibition of membrane ruffling) and two *in vitro* effects (an inhibition of actin filament elongation and an effect on viscosity of actin filaments). The results indicated that there are almost the same hierarchic orders of relative effectiveness of different cytochalasans between low and high dose effects and between cellular and molecular effects. The strong positive correlation suggests that most of

the effects caused by a cytochalasan might be attributed to the interaction between the drug and the common target protein, actin. To clarify the minimal structural characteristics required for the drug effects not mentioned in the previous paper,²⁾ three derivatives were newly prepared from chaetoglobosin A as shown below. The effects of thirty-nine compounds, twenty natural compounds reported in the previous paper, three new member of the group, seven derivatives prepared, and nine partial synthones of cytochalasin B, were compared to investigate the structure-activity relationship to supplement the previous work.²⁾

MATERIALS AND METHODS

Natural Cytochalasans — The name of cytochalasans are generally used in the abbreviated forms as shown in Tables I and II. The structures are shown in Table I.³⁻⁵⁾ The source of the samples were shown in the previous paper.²⁾ CK, CL,

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and CM are the metabolites of *Chalara microspora*⁶⁾ and the samples generously provided by Dr. T. Fex (Lund Institute of Technology, Lund, Sweden) were newly added in this work. Thus, twenty-three natural cytochalasans among forty so far known³⁻⁵⁾ were examined in this work.

Derivatives of Chaetoglobosins—Besides the four acetates of chaetoglobosins,⁷⁻⁹⁾ three derivatives of chaetoglobosin A were prepared as follows. The structures (Table I) of the products were mainly confirmed by the comparison of ¹H-^{8,9)} and ¹³C- nuclear magnetic resonance¹⁰⁾ (NMR) data with those reported.

21,22-Dihydrochaetoglobosin B (Dihydro-ChB): A solution of ChA (128 mg) in EtOH (36 ml) was shaken under hydrogen in the presence of palladized charcoal (36 mg) for 20 h. The product was applied to a silica gel column and elution with CHCl₃-MeOH (20:1) gave a solid, which was recrystallized from EtOH to afford colorless needles of mp 251–252.5 °C. MS *m/z*: 530 (M⁺, C₃₂H₃₈N₂O₅), 130 (base peak). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3540, 3460, 3355, 2950, 2900, 1700, 1675, 1610, 1442, 1420, 1250, 1042, 986, 982, 865, 743. ¹H-NMR* (*d*₆-DMSO) δ : 0.91 (3H, d, *J*=6.8 Hz, C₁₆-CH₃), 1.42 (3H, s, C₆-CH₃), 1.54 (3H, s, C₁₈-CH₃), 1.61 (3H, s, C₅-CH₃), 1.86 (2H, m, C₂₁-H₂), 2.12 (1H, dd, *J*=10.4, 9.8 Hz, C₈-H), 2.22 (1H, m, C₁₆-H), 2.72 (1H, dd, *J*=15.0, 5.0 Hz, C₁₀-H), 3.00 (1H, d, *J*=3.0 Hz, C₄-H), 3.62 (1H, m, C₃-H), 4.51 (1H, d, *J*=9.8 Hz, C₇-H), 5.03 (1H, m, C₁₄-H), 5.30 (1H, s, C₁₉-H), 5.33 (d, *J*=10.0 Hz, C₁₇-H), 6.14 (1H, dd, *J*=14.7, 10.4 Hz, C₁₃-H), 7.02 (1H, br s, C₂-H), 7.3–7.5 (4H, m, 4',5',6',7'-H₄), 8.07 (1H, br s, 2-NH), 10.63 (1H, br s, 1'-NH). ** ¹³C-NMR* (*d*₆-DMSO) δ : 10.8 (C₁₈-CH₃), 14.4 (C₅-CH₃), 16.7 (C₆-CH₃), 21.0 (C₁₆-CH₃), 31.6 (C₁₀), 31.8 (C₁₆), 33.4 (C₂₁***), 34.7 (C₂₂***), 40.9 (C₁₅), 48.8 (C₈), 51.2 (C₃), 57.5 (C₄), 61.8 (C₉), 67.7 (C₇), 82.0 (C₁₉), 109.7 (C₃'), 111.4 (C₇'), 117.8 (C₄'), 118.3 (C₆'), 120.8

(C₂'), 123.6 (C₅'), 125.4 (C₅), 127.0 (C₃'a), 128.2 (C₁₃), 131.6 (C₁₈), 133.5 (C₁₄***), 133.6 (C₆***), 136.0 (C₁'a), 137.2 (C₁₇), 173.3 (C₁), 209.2 (C₂₀***), 209.6 (C₂₃***).

Chaetoglobosin A Imino Methyl Ether 19-O, 1'-N-Dimethyl Ether (ChA-triMe): To the solution of ChA (100 mg) in dimethylformamide (2 ml) and CH₃I (2 ml) was added Ag₂O (200 mg) under dark condition and the reaction mixture was left standing for 24 h. After filtration, water was added and chloroform extract of the reaction mixture was passed through a silica gel column. The trimethyl ether, mp 117–118 °C, was obtained from the CHCl₃-AcOEt (3:2) eluate. MS *m/z*: 570 (M⁺, C₃₅H₄₂N₂O₅), 144 (*N*-methyl-indolylmethyl, base peak). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3520, 3480, 3420, 3080, 3060, 2950, 2920, 2860, 1720–1650, 1600, 1490, 970, 900, 740, 700. ¹H-NMR (CDCl₃) δ : 0.81 (3H, d, *J*=6.8 Hz, C₁₆-CH₃), 1.03 (3H, d, *J*=4.5 Hz, C₅-CH₃), 1.20 (3H, s, C₆-CH₃), 1.44 (3H, s, C₁₈-CH₃), 1.75 (1H, dq, *J*=5.8, 4.3 Hz, C₅-H), 1.86 (2H, m, C₁₅-H₂), 2.25 (1H, dd, *J*=4.4, 9.9 Hz, C₈-H), 2.55 (1H, m, C₁₆-H), 2.63 (2H, m, C₇-H and C_{10a}-H), 2.94 (1H, dd, *J*=15.0, 4.0 Hz, C_{10b}-H), 3.34, 3.73, 3.96 (each 3H, s, -CH₃ × 3), *⁴ 4.15 (1H, m, C₃-H), 4.43 (1H, s, C₁₉-H), 5.18 (1H, m, C₁₄-H), 5.36 (1H, d, *J*=9.2 Hz, C₁₇-H), 5.75 (1H, dd, *J*=9.9, 15.6 Hz, C₁₃-H), 6.55 (1H, d, *J*=15.2 Hz, C₂₂-H), 6.75 (1H, s, C₂-H), 7.0–7.2 (4H, m, 4',5',6',7'-H₄), 7.32 (1H, d, *J*=15.2 Hz, C₂₁-H), no NH signal. ¹³C-NMR (CDCl₃) δ : 10.7 (C₁₈-CH₃), 12.6 (C₅-CH₃), 19.5 (C₆-CH₃), 21.1 (C₁₆-CH₃), 32.7 (N₁'-CH₃), 32.8 (C₁₀, C₁₆), 36.5 (C₅), 40.7 (C₁₅), 47.2 (C₄), 50.6 (C₈), 55.8 (C₃), 56.3 (C₁₉-OCH₃), 57.8 (C₆), 61.5 (C₇), 65.3 (C₁-OCH₃), 67.9 (C₉), 91.5 (C₁₉), 109.4 (C₇'), 110.1 (C₃'), 118.8 (C₄'), 119.5 (C₆'), 121.7 (C₂'), 126.9 (C₅'), 127.7 (C₃'a), 128.1 (C₁₃), 130.8 (C₁₈), 133.6 (C₂₁***), 134.7 (C₁₄***), 135.5 (C₂₂***), 136.7 (C₁'a), 141.1 (C₁₇), 168.5 (C₁), 196.6 (C₂₃***), 200.2 (C₂₀***). The spectral data in-

* The NMR spectra were run on a JEOL FX-200 machine (¹³C, 50.1 MHz).

** C_{10b}-H, C₁₅-H₂ and C₂₂-H₂ were not assigned due to overlapping with the solvent and water signals.

*** The assignments may be reversed.

**** C₄-H: overlapping with the methyl signals.

indicated that the product is imino methyl ether of ChA dimethyl ether.¹¹⁾

Lithium Aluminum Hydride Reduction Product of Chaetoglobosin A (TetrahydroChA): A solution of ChA (200 mg) in anhydrous tetrahydrofuran was added to a solution of LiAlH_4 in anhydrous tetrahydrofuran (0.1 M, 10 ml) at room temperature. After 15 h 10% H_2SO_4 (20 ml) was added to the reaction mixture. The EtOAc extract was applied to preparative thin-layer chromatography on silica gel plates and the main product was isolated by development with EtOAc. mp 265 °C, MS m/z : 532 (M^+ , $\text{C}_{32}\text{H}_{40}\text{N}_2\text{O}_5$), 130 (base peak). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300 (br), 2950, 2910, 1665 (br), 1606, 1435, 1380, 1338, 1280, 1230, 1040, 972, 875, 740. ^1H -NMR (d_6 -DMSO) δ : 0.85 (3H, d, $J=6.6$ Hz, $\text{C}_{16}\text{-CH}_3$), 0.98 (3H, d, $J=6.9$ Hz, $\text{C}_5\text{-CH}_3$), 1.18 (3H, s, $\text{C}_6\text{-CH}_3$), 1.50 (3H, s, $\text{C}_{18}\text{-CH}_3$), 1.52 (1H, dq, $J=6.9, 5.0$ Hz, $\text{C}_5\text{-H}$), 1.71 (2H, m, $\text{C}_{15}\text{-H}_2$), 1.87 (1H, dd, $J=10.0, 5.5$ Hz, $\text{C}_8\text{-H}$), 2.10 (1H, dd, $J=5.2, 5.0$ Hz, $\text{C}_4\text{-H}$), 2.36 (1H, m, $\text{C}_{16}\text{-H}$), 2.63 (1H, d, $J=5.5$ Hz, $\text{C}_7\text{-H}$), 2.80 (1H, dd, $J=14.0, 4.0$ Hz, $\text{C}_{10a}\text{-H}$), 3.71 (1H, m, $\text{C}_3\text{-H}$), 4.00 (1H, m, $\text{C}_{20}\text{-H}$), 4.63 (1H, d, $J=5.1$ Hz, $\text{C}_{19}\text{-H}$), 4.79 (1H, d, $J=6.9$ Hz, $\text{C}_{17}\text{-H}$), 4.81 (1H, m, $\text{C}_{14}\text{-H}$), 5.92 (1H, dd, $J=15.1, 10.0$ Hz, $\text{C}_{13}\text{-H}$), 6.99 (1H, br s, $\text{C}_2'\text{-H}$), 7.0–7.5 (4H, m, $\text{C}_4'\text{-C}_7'\text{-H}_4$), 8.18 (1H, br s, 2-NH), 10.89 (1H, br s, 1'-NH).^{*} ^{13}C -NMR (d_6 -DMSO) δ : 12.2 ($\text{C}_5\text{-CH}_3^{**}$), 12.3 ($\text{C}_{18}\text{-CH}_3^{**}$), 19.1 ($\text{C}_6\text{-CH}_3$), 21.0 ($\text{C}_{16}\text{-CH}_3$), 26.6 (C_{21}^{**}), 30.3 (C_{16}), 32.1 (C_{10}), 36.2 (C_5 and C_{22}^{**}), 41.6 (C_{15}), 48.0 (C_8), 48.9 (C_4), 51.7 (C_3), 56.7 (C_6), 61.1 (C_7), 63.1 (C_9), 71.0 (C_{19}^{**}), 77.3 (C_{20}^{**}), 107.9 (C_3'), 111.0 (C_7'), 118.3 (C_4' and C_6'), 120.6, (C_2'), 125.0 (C_5'), 127.6 ($\text{C}_3'a$), 129.1 (C_{13}), 131.3 (C_{18}), 133.2 (C_{14}), 134.1 (C_{17}), 135.9 ($\text{C}_{1'a}$), 173.3 (C_1), 209.4 (C_{23}).

Partial Synthones of Cytochalasins — Nine partial synthones (Chart 1) were prepared by Professor G. Stork, Dr. W. J. Greenlee (present address, Merck & Co., Rahway, N. J.) and Dr. E.

Nakamura (present address, Tokyo Institute of Technology, Tokyo) at the Department of Chemistry, Columbia University, New York, N. Y., in the course of study on the total synthesis of CB¹²⁾ and the synthones were generously provided for this work.

Cell Morphology — An established mouse cell line, C3H-2K, was generally used for determination of effects of cytochalasins on cell morphology. C3H-2K cells were cultured in Dulbecco's modified Eagle medium-10% fetal calf serum (FCS) containing 100 $\mu\text{g}/\text{ml}$ Kanamycin sulfate (Meiji Seika Kaisha Ltd., Tokyo). Confluent cultures of C3H-2K cells were trypsinized and plated in culture dishes as described, and cultured at 37 °C for 4 h during which the cells spread over the substrate to a considerable degree. Cytochalasins were added when indicated and the effects of the drugs on cell morphology were determined 30 min later.

Immunofluorescence Observations of Cellular Actin — The distribution of actin in untreated and cytochalasin-treated C3H-2K cells was determined by the indirect immunofluorescence method with rabbit anti-chick embryonic fibroblast actin antibody²⁾ or rabbit anti-chick gizzard actin antibody.¹³⁾ The method for staining was previously described.²⁾

Lymphocyte Capping — Splenic lymphocytes of DBA/2 mice (Charles River, Tokyo, Japan) were used for capping experiments. All the procedures were previously described in detail.¹⁴⁾

Actin Filament Elongation — Purified rabbit skeletal muscle actin (1 mg/ml) was fully polymerized in buffer A (2 mM Tris-HCl, pH 8.0, 0.2 mM adenosine triphosphate (ATP), 0.5 mM β -mercaptoethanol, 0.2 mM CaCl_2) containing 2 mM MgCl_2 . Two ml of actin monomer solution (1 mg/ml) in buffer A were mixed with 50 μl of fully polymerized actin solution and 20 μl of 0.05 M MgCl_2 solution. Cytochalasins were dissolved dimethylsulfoxide (DMSO) at 0.2 mM, and were added at 2 μM to the actin monomer

* $\text{C}_{10b}\text{-H}$, $\text{C}_{21}\text{-H}_2$, and $\text{C}_{22}\text{-H}_2$ signals are overlapping with the solvent and water signals.

** The assignments may be reversed.

solution just before the addition of polymerized actin and MgCl_2 . DMSO alone in 1% (v/v), as a control, did not affect actin polymerization at all. Actin polymerization was monitored up to 40 min by a viscometric method previously described.²⁾

Lymphocyte Stimulation — Cultures of DBA/2 mouse splenic lymphocytes (6×10^5 cells/0.2 ml/culture well) first received cytochalasans at 0.2, 2 or 20 μM and subsequently concanavalin A (Con A) at 2 $\mu\text{g/ml}$. The cultures were incubated in a CO_2 incubator (5% CO_2 in the air) at 37 °C for 2 d. ^3H -Thymidine (5 $\mu\text{Ci/culture}$) was added and the cultures were incubated for another 6 h. The cells were harvested on Whatman GF/A glass filters and trichloroacetic acid-insoluble radioactivities were determined with a liquid scintillation counter.

RESULTS

Alteration in Actin Distribution

Contraction and/or disintegration of stress fibers composed of hundreds of actin filaments were examined by the immunofluorescence method with anti-actin antibody. Cytochalasans at 20 μM were mostly used for treatment at 37 °C for 30 min. With a mild cytochalasan such as CB, treatment induced contraction of stress fibers followed by arborization of cell morphology. As previously noted, induction of hairy structures consisting of actin filaments were observed in association with the arborization.²⁾ With a strong cytochalasan such as ChA, treatment of C3H-2K cells at 20 μM rapidly induced cell rounding up so that contraction of stress fibers was not detected on the majority of treated cells. The contraction of stress fibers was detected on cells treated with 2 μM chaetoglobosin A at 37 °C for 30 min. The results obtained with various cytochalasans, a part of which had been reported in the previous paper,²⁾ were tabulated in Table II (column 1).

Alteration in Cell Morphology

C3H-2K cells were separately treated at 37 °C for 0.5 to 1 h with various cytochalasans at

three different concentrations. Cell morphology of these cells was monitored without fixation under a phase contrast microscope. When a drug at 2 μM barely induced rounding up of these cells within 1 h, but the same drug at 20 μM caused rounding up, the effect of the drug was expressed as +. When a drug was effective as low as 2 μM , the effect was expressed as ++. When rounding up of cell body was not induced at any dose up to 20 μM within 1 h, the effect was expressed as -. The results are shown in column 2 of Table II.

Inhibition of Lymphocyte Capping

Mouse splenic lymphocytes suspended in phosphate-buffered saline (PBS) were incubated at 37 °C for 10 min with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin (FITC-R-anti-MIg) in the presence of various cytochalasans at 20 μM . Cytochalasans were generally added to the cell suspensions 10 min prior to the start of the incubation with FITC-R-anti-MIg. The extent to which capping was inhibited by the drugs is shown in column 3 of Table II.

Inhibition of Actin Filament Elongation

To assess the high affinity effect of cytochalasans *in vitro*, we have determined effects of various cytochalasans at 2 μM on actin filament elongation. When a small amount of polymerized actin was added as polymerization nuclei, elongation of actin filaments as monitored by viscometry started without any lag period, then proceeded almost linearly up to 20 min, and declined thereafter. The polymerization did not reach a plateau within 40 min. An increase in specific viscosity per min, which was obtained on the average between 10 and 20 min, was 0.0152 min^{-1} for the control mixture with no inhibitor.²⁾ This value reveals the rate of actin filament elongation in this system. The rate of actin filament elongation was also determined in the presence of cytochalasans. Reduction in the rate as expressed % of the control rate was determined for different cytochalasans (Table II, column 4).

Inhibition of Lymphocyte Stimulation

Stimulation of resting T cells by Con A is divided into three separable steps; (i) production of interleukin 2 (IL2), a growth factor for T cells; (ii) emergence of IL2 receptors on the surfaces of T cells to be stimulated; (iii) binding of IL2 to its receptors and induction of a series of events leading to the initiation of deoxyribonucleic acid (DNA) synthesis.¹⁵⁾ We have previously observed that CB or CD interfered with process (i) but not the processes (ii) or (iii) (unpublished observation). However, for the rapid assessment of inhibitory effects of various cytochalasans on process (i), we measured in the present study the overall reactions by determining incorporation of ³H-thymidine into trichloroacetic acid (TCA)-precipitable fractions after resting lymphocytes were incubated with 2 μ g/ml Con A. Triplicate cultures were prepared

for each determination shown in Table III and the values were averages of the three. DMSO was added to control cultures at 0.1% (v/v) because the drugs were dissolved in DMSO. Cytochalasans did not affect Con A itself because of the following experiment. When Con A was mixed with either one of the three cytochalasans used in this experiment and recovered from the mixtures by gel filtration through a Sephadex G-100 column, the activity of Con A to stimulate T cells was found to remain without any loss. Each value was obtained by reduction of cpm incorporated into unstimulated cultures (— Con A) from cpm incorporated into stimulated cultures (+ Con A). All the three cytochalasans tested were found to inhibit the stimulation of mouse T cells by Con A in a dose-dependent manner (Table III).

TABLE I. *The Structures of Cytochalasans and the Derivatives Tested*

	R ²	R ³
10-Phenylcytochalasans (R ¹ : phenyl)		
Cytochalasin A (CA)
Cytochalasin B (CB)	Same as CA
Cytochalasin C (CC)
Cytochalasin D (CD)	Same as CA	Same as CC
Cytochalasin E (CE)
Cytochalasin H (CH)	Same as CA
Cytochalasin J (CJ)	Same as CA	Same as CH (R: H)
Cytochalasin K (CK)	Same as CE
Cytochalasin L (CL)	Same as CE

Cytochalasin M (CM)	Same as CE
Deoxaphomin	Same as CA
10-(Indol-3-yl)cytochalasans (R ¹ : indol-3-yl)		
Cytochalasin G (CG)	Same as CE
Chaetoglobosin A (ChA)	Same as CE	Same as CK (R: H)
Chaetoglobosin B (ChB)	Same as CC	Same as CK (R: H)
Chaetoglobosin C (ChC)	Same as CE
Chaetoglobosin D (ChD)	Same as CA	Same as CK (R: H)
Chaetoglobosin E (ChE)	Same as CC
Chaetoglobosin F (ChF)	Same as CE	Same as ChE (R: H)
Chaetoglobosin J (ChJ)		Same as CK (R: H)
19- O-Acetylchaetoglobosin A (ChA-Ac)	Same as CE	Same as CK (R: Ac)
Chaetoglobosin K (ChK)	Same as CE (10-Me, 11-Me)	Same as CK (R: H)
10-Isopropylcytochalasans (R ¹ : isopropyl)		
Aspochalasin B (AsB)	Same as ChJ
Aspochalasin D (AsD)	Same as ChJ
Chaetoglobosin derivatives prepared (R ¹ : indol-3-yl)		
7,19- O-Diacetylchaetoglobosin D (ChD-Ac)		Same as CK (R: Ac)
7,19- O-Diacetylchaetoglobosin E (ChE-Ac)		Same as ChE (R: Ac)
19- O-Acetylchaetoglobosin F (ChF-Ac)	Same as CE	Same as ChE (R: Ac)
19- O-Acetylchaetoglobosin J (ChJ-Ac)	Same as ChJ	Same as CK (R: Ac)
21,22-Dihydrochaetoglobosin B (dihydroChB)	Same as CC

Chaetoglobosin A
imino methyl ether
19-*O*,1'-*N*-dimethyl
ether (ChA-triMe)

Same as CE

Same as CK (R: Me)

20,20,21,22-
Tetrahydrochaeto-
globosin A
(tetrahydroChA)

Same as CE

CHART 1. *The Structures of Synthetic Synthones*

TABLE II. *Effects of Cytochalasans*

Drugs (Abbreviation)	(1) Actin-cables contraction	(2) Cell rounding up	(3) Inhibition of capping (%)	(4) Inhibi- tion of polymeri- zation (%)
10-Phenylcytochalasans				
Cytochalasin A (CA)	+	++	95	42
Cytochalasin B (CB)	+	+	47	31
Cytochalasin C (CC)	+	++	64	46
Cytochalasin D (CD)	+	++	79	72
Cytochalasin E (CE)	+	++	94	61
Cytochalasin H (CH)	+	++	73	51
Cytochalasin J (CJ)	+	+	41	45
Cytochalasin K (CK)	±	+	98	39
Cytochalasin L (CL)	±	+	93	35
Cytochalasin M (CM)	+	++	33	12
Deoxaphomin	+	+	70	38

10-(Indol-3-yl)cytochalasans				
Cytochalasin G (CG)	+	+	27	22
Chaetoglobosin A (ChA)	+	+++	98	50
Chaetoglobosin B (ChB)	+	+++	93	42
Chaetoglobosin C (ChC)	+	+	59	14
Chaetoglobosin D (ChD)	+	+++	93	62
Chaetoglobosin E (ChE)	+	+	49	22
Chaetoglobosin F (ChF)	+	+	67	23
Chaetoglobosin J (ChJ)	+	+++	90	54
19- <i>O</i> -Acetylchaetoglobosin A (ChA-Ac)	+	+++	97	50
Chaetoglobosin K (ChK)	—	—	93	51
10-Isopropylcytochalasans				
Aspochalasin B (AsB)	+	+	39	0
Aspochalasin D (AsD)	+	+	60	5
Chaetoglobosin derivatives prepared				
7,19- <i>O</i> -Diacetylchaetoglobosin D (ChD-Ac)	+	++	60	19
7,19- <i>O</i> -Diacetylchaetoglobosin E (ChE-Ac)	—	—	20	5
19- <i>O</i> -Acetylchaetoglobosin F (ChF-Ac)	±	±	24	26
19- <i>O</i> -Acetylchaetoglobosin J (ChJ-Ac)	+	++	79	38
21,22-Dihydrochaetoglobosin B (dihydroChB)	±	±	27	15
Chaetoglobosin A iminomethyl ether 19- <i>O</i> -,1'- <i>N</i> -dimethyl ether (ChA-triMe)	+	+++	59	21
20,20,21,22-Tetrahydrochaetoglobosin A (tetrahydroChA)	+	++	41	17
Synthetic symthones 1–9 (Chart 1)	—	—	0	0

TABLE III. *Effect of Cytochalasans on Lymphocyte Stimulation*

		³ H-Thimidine incorporation cpm (+ Con A) – cpm (– Con A)	Inhibition (%)
0.1% DMSO		610 000	0
CB	0.2 μM	618 000	0
CB	2 μM	285 000	53
CB	20 μM	20 000	97
CD	0.2 μM	459 000	36
CD	2 μM	43 200	94
CD	20 μM	1 200	100
ChA	0.2 μM	610 000	15
ChA	2 μM	144 000	80
ChA	20 μM	200	100

DISCUSSION

The common structural feature of the group of mycotoxins called cytochalasans is expressed by a perhydroisoindol-1-one bearing a benzyl, indol-3-ylmethyl or 2-methylpropyl group at the C₃ position and a 11-, 13-, or 14-membered carbocyclic (or oxygen-containing) ring between the C₈ and C₉ positions.^{1,3-5)} The structure-activity relationship observed in the cytotoxicity of more than thirty cytochalasin D derivatives was reported and the free hydroxyl group at C₇ and the phenyl group at C₁₀ were claimed to be essential to the manifestation of the toxicity.¹⁶⁾ As reported in the previous paper¹⁷⁾ the cytotoxicity to HeLa cells of chaetoglobosins bearing the indol-3-yl group at C₁₀ demonstrated that phenyl group is replaceable by another aromatic ring.

As shown in our previous paper,²⁾ the most of the effects caused by cytochalasans might be attributed to the interaction between the drug and common target protein, actin. The effects *in vitro* and those *in vivo* showed good correlation. This fact was further confirmed in this work. The correlation between inhibition of capping and inhibition of actin filament elongation observed in thirty compounds listed in Table II is shown in Fig. 1.

Since the structure-activity relationship was not discussed in the previous paper,²⁾ details will be given as follows: The substituents at C₁₀, the cellular events and the *in vitro* effects were in the same order in the phenyl and indolyl series. For example, CK and ChA-Ac, in which the structures were entirely the same except the C₁₀ substituents, showed the same order of effects. When the group was replaced by isopropyl group as AsB and AsD, the same effects were observed though the *in vitro* effect was quite weak. Considering of the results obtained by the Shionogi group,¹⁶⁾ a conclusion can be drawn that the substituents at C₁₀ influence the magnitude of the effects but a specific group is not required as a binding site.

The perhydroisoindol-3-one nucleus has been assumed to be the most important factor for the

manifestation of the contractile inhibition and the 1-CO, 2-NH, and C₇-OH functions were claimed, without any direct evidence, as the principal rigidly configured binding group.^{18,19)} Present work indicated that the compounds having an epoxide group at C₆-C₇ (CE, CK, CL, CM, CG, ChA, ChC, ChF, ChA-Ac and ChK) and those with no oxygen function (ChJ, AsB, AsD, and ChJ-Ac) also exhibit these effects. When the effects of the pairs of compounds, in which the structures of the whole molecules are the same except the functionalities at C₅, C₆, and C₇, *i.e.* CC and CD and ChA, ChB, ChD and ChJ, were compared, obvious difference was not observed. However, in the case of the compounds having a hydroxyl group at C₇ (ChD and ChE), acetylation of the group clearly rendered decrease of the activity. The trimethyl ether prepared from ChA (ChA-triMe), assigned as imino methyl ether of the lactam of ChA, retained the activity.

The macrocyclic ring starting at C₈ and running to C₉ shows variety in the size of the ring and the functionalities over C₁₇. In 10-phenylcytochalasans tested, the *in vivo* and *in vitro* effects are in the order of CA, CD, CE, CH, CK, CL > CC, deoxaphomin > CB, CJ, CM. The enlargement of the 13-membered ring to the 14-membered ring by the Baeyer-Villiger type insertion of an oxygen atom at the C₉ end did not influence the activity (CK and CL, deoxaphomin and CB). Thus, the size of the ring and the special functionalities did not correlated directly to the activity. In the case of 10-(indol-3-yl)cytochalasans, the order, ChA, ChB, ChD, ChJ, ChA-Ac, ChK > ChC, ChE, ChF, dihydro-ChB, tetrahydroChA > CG, indicate the enhancing effect of the group, 21-ene-20,23-dione. This argument is applicable in the case of 10-phenylcytochalasans as indicated by the comparison of CL and CM. However, the conjugated system is not assumed as obligatory for electrophilic interaction of the drug with presumptive receptor nucleophiles.^{19,20)} As a whole, no definite functional group in this part of the molecule has a role as a binding site with the contractile

protein. It is probable that the region from C₁₃ to C₁₆ common to almost all cytochalasans acts as an anchor in a hydrophobic domain.²¹⁾

In the cytotoxicity testing of the cytochalasin D derivatives, the cleavage of the macrocyclic ring retained the activity.¹⁶⁾ It was also reported that the γ -lactone formed by the cleavage of the ester bond between C₂₃ and C₂₄ of CB showed the binding activity with the site related to cell motility and morphology (site II) in erythrocyte membrane.²²⁾ However, all nine synthetic syntheses for cytochalasins (the structures shown in Chart 1)¹²⁾ did not show any activity in our systems.

ChK, AsB, and AsD are unique in the structures among the natural cytochalasans so far known³⁻⁵⁾ and tested in this work (Table I). ChK, having two extra methyl groups at C₁₀ and C₁₁, did not show the effect to actin cable, though it exhibited other effects. Aspochalasins, having an isopropyl group at C₁₀ instead of aromatic residues and bearing a methyl group at C₁₄ in place of C₁₆ in others, showed little effects

to actin polymerization. Thus, the cytochalasans with exceptional structures exhibit exceptional behaviour to the biological systems.

Using a gel-sol model obtained from Ehrlich ascites cell, which mimics actin-related manifestations of intact cells, Tanenbaum *et al.*¹⁹⁾ concluded that the C₇-oxygen atom, an unsaturated lactam group, and remnants of the macrocyclic moiety, including the C₁₃-C₁₄ region, are essential for the contractile inhibition. Rampal, *et al.*²²⁾ reported the structure-activity relationship observed in binding sites in human erythrocyte membrane: While the size and the structure of the macrocyclic ring and the nature of the aromatic ring at C₁₀ were assumed to modulate the effect slightly, the modification in C₅-C₇ region of the molecule results in substantial changes in the activity. They also mentioned that the effects observed by their systems are not parallel to the results using other cell systems. In the inhibition of mitogen-induced human lymphocyte proliferation, Mookerjee, *et al.*²³⁾ reported that the size and the substitution in the macrocyclic ring and the change of the substitution pattern in core cyclohexane ring are not critically influential but the 10-phenyl group is very important for determining the efficacy. However, our work on lymphocyte stimulation indicated that ChA showed the effect in a same order as CB and CD (Table III) contrary to their argument.

As a whole, the core composed of the perhydroisoindolone and the macrocyclic ring and the hydrophobic region from C₁₃ to C₁₆ are assumed to be essential for the effects. The aromatic ring, the units over C₁₆ of the macrocyclic ring including a conjugated enedione system, and the physical properties of the compounds as a whole such as the lipophilicity are influential in their relative effectiveness. Further work is necessary to clarify this point.

FIG. 1. Correlation between Inhibition of Capping and Inhibition of Actin Filament Elongation by Thirty Cytochalasans

Nine synthetic syntheses with no effect were excluded.

●, 10-phenylcytochalasans; ○, 10-indolylcytochalasans; □, 10-isopropylcytochalasans; ■, chaetoglobosin derivatives prepared.

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