The Effects of New Cytochalasins from *Phomopsis* sp. and the Derivatives on Cellular Structure and Actin Polymerization

Takashi HIROSE,[a] Yoshihiko IZAWA,[a] Kiyotaka KOYAMA,[a] Shinsaku NATORI,*[,a] Kazuko IIDA,[b] Ichiro YAHARA,[b] Shin SHIMAOKA[c] and Koscak MARUYAMA[c]

Meiji College of Pharmacy,[a] Yato-cho, Tanashi, Tokyo 188, Japan, The Tokyo Metropolitan Institute of Medical Science,[b] Honkomagome-3-chome, Bunkyo-ku, Tokyo 113, Japan and Department of Biology, Faculty of Science, Chiba University,[c] Chiba 260, Japan. *Received August 8, 1989*

The effects of ten 10-phenyl-[11]cytochalasans produced by *Phomopsis* sp. including novel compounds having 5,7- or 6,7-glycol structures and their derivatives, on the cell morphology and actin distribution in C3H-2K cells, as well as on lymphocyte capping and actin polymerization, were examined. The structure–activity relationship reported in the previous papers has been confirmed. The novel glycol type compounds showed little or no activity, suggesting the importance of the perhydroisoindol-1-one nucleus for the manifestation of the cytochalasin actions.

Keywords cytochalasan; cytochalasin; *Phomopsis* sp.; C3H-2K cell; cellular structure; lymphocyte capping; actin polymerization; structure–activity relationship

The cytochalasins[1] are fungal secondary metabolites, which inhibit a variety of cellular movements, including cell division and motility, and cause changes in cell shape. In the previous papers,[2,3] the effects of twenty-three natural cytochalasins and the sixteen derivatives on actin-distribution (contraction of stress fibers) and on the morphology of C3H-2K cells, lymphocyte capping, and actin filament elongation were compared. The effects caused by cytochalasins were proved to be attributable to the interactions between the drugs and the common target protein, actin, and the structure–activity relationship was discussed. Quite recently we have isolated ten 10-phenyl-[11]cytochalasans[1] (**1**—**10**) from *Phomopsis* sp. (68-GO-164), including six new compounds named cytochalasins N—S (**5**—**10**).[4] Since some of the new compounds have novel diol-type structures in the perhydroisoindol-3-one moiety, which is assumed to be essential for the activities,[3] it seemed worthwhile to examine their cellular effects. As pointed out in the previous paper,[3] the physical properties of the compounds as a whole such as the lipophilicity, influence the relative effectiveness. Thus, five derivatives (**11**—**15**) were prepared to clarify this point. This paper concerns the effects of these compounds on cellular structures, lymphocyte capping and actin polymerization.

Materials and Methods

Natural Cytochalasins (1—10, 16) Four known cytochalasins, epoxycytochalasins H (**1**) and J (**2**) and cytochalasins H (**3**) and J (**4**), and six new compounds, cytochalasins N—S (**5**—**10**) were isolated in our laboratory as metabolites of *Phomopsis* sp. (68-GO-164).[4] Pyrichalasin H (**16**) was isolated as a phytotoxic metabolite of *Pyricularia grisea*[5] and the sample was kindly provided by Dr. M. Nukina. The structures of these compounds are shown in Chart 1. Cytochalasin D and chaetoglobosin A, used as the controls, were the same samples used in the previous studies.[2,3]

Derivatives of Cytochalasins (11—15) The following derivatives were prepared to elucidate the structure–activity relationship. The structures of the synthetic samples were confirmed by the physical data especially by mass spectra (MS) and the acylation shifts observed in ^{1}H and ^{13}C nuclear magnetic resonance (^{1}H and ^{13}C-NMR) spectra as compared with the spectra of the natural products.[4] The NMR spectra were recorded on a JEOL GX-400 spectrometer with tetramethylsilane as an internal standard.

21-*O*-Octanoylepoxycytochalasin J (11) Epoxycytochalasin J (**2**) (120 mg) was treated with pyridine (2.0 ml) and octanoyl anhydride (2.0 ml) for 3 h at room temperature under stirring and the precipitate formed by the addition of the reaction mixture to water was extracted with CH_2Cl_2. The residue, after evaporation of the solvent, was purified by high performance liquid chromatography (HPLC) on a Nucleosil 50-5 column employing hexane–acetone (5:2) as the developer to give the octanoyl ester (**11**) (92 mg), colorless powder, mp 93—95 °C (hexane–acetone). $[\alpha]_D^{23}$ +14.4° (c=0.20, MeOH). UV λ_{max}^{MeOH} nm (ε): 206 (17260). IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 2850, 1735, 1690, 1450, 1370, 1160, 1100, 960, 700. MS *m/z*: 577.3772 (M$^+$, Calcd for $C_{36}H_{51}NO_5$, 577.3770), 559, 541, 433, 415, 397, 324, 306, 251, 240, 120, 91, 60, 43. ^{1}H-NMR (DMSO-d_6) δ: 0.243 (d, J=7.2 Hz, 5-CH$_3$), 0.842 (t, acyl-CH$_3$), 0.962 (d, J=6.3 Hz, 16-CH$_3$), 1.074 (s, 6-CH$_3$), 1.175 (s, 18-CH$_3$), 1.22—1.26 (m, acyl-CH$_2$ × 5), *ca.* 1.42 (m, 17b-H), *ca.* 1.48 (m, 5-H), *ca.* 1.60 (m, 17a-H), *ca.* 1.62 (m, 15b-H), *ca.* 1.66 (m, 16-H), *ca.* 1.91 (m, 15a-H), *ca.* 1.91 (m, 4-H), *ca.* 2.42 (m, 8-H), *ca.* 2.42 (m, acyl-CH$_2$), 2.606 (d, J=5.5 Hz, 7-H), 2.996 (dd, J=3.9, 12.6 Hz, 10a-H), 3.524 (m, 3-H), 3.610 (m, 10b-H), 5.151 (ddd, J=5.2, 10.2, 15.4 Hz, 14-H), 5.385 (dd, J=2.2, 16.8 Hz, 20-H), 5.590 (d, J=2.2 Hz, 21-H), 5.683 (dd, J=2.2, 16.8 Hz, 19-H), 5.820 (dd, J=9.9, 15.4 Hz, 13-H), 7.20—7.30 (m, arom-H), 8.316 (s, 2-NH). ^{13}C-NMR (DMSO-d_6) δ: 11.7 (5-CH$_3$), 13.8, 22.0, 24.9, 28.3, 28.4, 30.9, 33.6 (21-OCO(CH$_2$)$_6$CH$_3$), 19.0 (6-CH$_3$), 25.9 (16-CH$_3$), 27.4 (16-C), 30.3 (18-CH$_3$), 35.9 (5-C), 42.4 (15-C), 44.7 (8-C), 44.8 (10-C), 48.1 (4-C), 53.0 (3-C), 53.5 (17-C), 53.6 (9-C), 56.7 (6-C), 62.1 (7-C), 72.0 (18-C), 75.0 (21-C), 124.4 (19-C), 126.4 (2′,6′-C), 128.2 (3′,5′-C), 128.4 (13-C), 129.6 (4′-C), 136.9 (1′-C), 138.6 (20-C), 140.0 (14-C), 172.3 (21-acyl-CO). 174.0 (1-C).

21-*O*-Octanoylcytochalasin O (12) A 1 N H_2SO_4 solution (1.5 ml) was added dropwise to a solution of the octanoyl ester (**11**) of epoxycytochalasin J (70.6 mg) in dimethyl sulfoxide (DMSO) (6.0 ml), and the solution was stirred for 30 min at room temperature.[4] The reaction mixture was neutralized by the addition of 10% $NaHCO_3$ and extracted with ether and the extract was purified by HPLC under the same conditions as above to give the octanoyl ester of cytochalasin O (**12**) (51.6 mg), colorless powder, mp 210—212 °C (hexane–acetone). $[\alpha]_D^{23}$ +62.9° (c=0.20, MeOH). UV λ_{max}^{MeOH} nm (ε): 206 (18530). IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 2850, 1725, 1697, 1445, 1262, 1150, 1100, 1030, 1000, 960, 700. MS *m/z*: 577.3751 (M$^+$, Calcd for $C_{36}H_{51}NO_5$, 577.3770), 559, 503, 486, 468, 434, 415, 360, 342, 324, 306, 296, 268, 251, 240, 157, 133, 120, 105, 91, 57, 43. ^{1}H-NMR (DMSO-d_6) δ: 0.807 (s, 6-CH$_3$), 0.838 (t, acyl-CH$_3$), 0.954 (d, J=6.1 Hz, 16-CH$_3$), 1.175 (s, 18-CH$_3$), *ca.* 1.2—1.4 (m, acyl-CH$_2$ × 5), *ca.* 1.42 (m, 17b-H), 1.498 (s, 5-CH$_3$), *ca.* 1.65 (m, 15b-H), *ca.* 1.65 (m, 17a-H), *ca.* 1.66 (m, 16-H), 1.89 (m, 15a-H), 2.244 (s, 4-H), 2.344 (dd, J=9.9, 9.9 Hz, 8-H), 2.510 (t, acyl-CH$_2$), 2.690 (dd, J=4.1, 12.9 Hz, 10a-H), 2.977 (dd, J=11.5, 12.9 Hz, 10b-H), 3.164 (m, 3-H), 3.606 (d, J=9.9 Hz, 7-H), 4.187 (d, 7-OH), 4.392 (s, 18-OH), 5.055 (ddd, J=5.2, 10.2, 15.4 Hz, 14-H), 5.392 (dd, J=1.9, 16.5 Hz, 20-H), 5.612 (dd, J=1.7, 16.5 Hz, 19-H), 5.688 (d, J=1.9 Hz, 21-H), 5.724 (dd, J=9.9, 15.4 Hz, 13-H), 7.22—7.30 (m, arom.-H), 8.170 (s, 2-NH). ^{13}C-NMR (DMSO-d_6) δ: 13.8, 22.0, 24.9, 28.4, 28.5, 31.0, 33.8 (21-OCO(CH$_2$)$_6$CH$_3$), 14.1 (5-CH$_3$), 16.2 (6-CH$_3$), 26.0 (16-CH$_3$), 27.5 (16-C), 30.3 (18-CH$_3$), 43.0 (15-C), 43.7 (10-C), 48.1 (8-C), 49.1 (3-C), 51.3 (9-C), 53.9 (17-C), 59.8 (4-C), 68.0 (7-C), 72.1 (18-C), 74.6 (21-C), 124.8 (19-C), 125.2 (5-C), 126.5 (4′-C), 128.3 (3′,5′-C), 128.8 (13-C), 129.3 (2′,6′-C), 133.2 (14-C), 133.9 (6-C), 137.5 (1′-C), 138.6 (20-C), 172.6 (21-acyl-CO), 174.2 (1-C).

7,21-*O*-Dioctanoylcytochalasin O (13) The diester (**13**) (51 mg) was prepared from cytochalasin O (51 mg) (**6**) by the same procedure as used for the preparation of **11**, colorless needles, mp 66—68 °C (hexane–

acetone). $[\alpha]_D^{23}$ +75.12° (c=0.21, MeOH). UV λ_{max}^{MeOH} nm (ε): 213 (13240). IR ν_{max}^{KBr} cm^{-1}: 3400, 2925, 2850, 1735, 1690, 1450, 1370, 1160, 1100, 960, 700. MS m/z: 703.4776 (M^+, Calcd for $C_{44}H_{65}NO_6$, 703.4810). ^{1}H-NMR (DMSO-d_6) δ: 0.80—0.90 (acyl-CH_3 × 2), 0.840 (s, 6-CH_3), 0.930 (d, J= 6.9 Hz, 16-CH_3), 1.12—1.34 (m, acyl-CH_2 × 10), 1.174 (s, 18-CH_3), 1.359 (s, 11-H), *ca.* 1.42 (m, 17b-H), *ca.* 1.54 (m, 15b-H), *ca.* 1.54 (m, 17a-H), *ca.* 1.67 (m, 16-H), *ca.* 1.82 (m, 15a-H), 2.02—2.22 (acyl-CH_2), 2.315 (s, 4-H), 2.50—2.60 (acyl-CH_2), *ca.* 2.52 (m, 8-H), 2.721 (dd, J=11.0, 12.9 Hz, 10b-H), 3.011 (dd, J=4.1, 12.9 Hz, 10a-H), 3.22 (m, 3-H), 5.076 (ddd, J=4.7, 10.5, 15.4 Hz, 14-H), 5.180 (d, J=9.4 Hz, 7-H), 5.418 (dd, J=1.9, 16.5 Hz, 20-H), 5.557 (dd, J=1.7, 16.5 Hz, 19-H), 5.630 (dd, J=10.2, 15.4 Hz, 13-H), 5.724 (d, J=1.9 Hz, 21-H), 7.24—7.32 (arom.-H), 8.325 (s, 2-NH). ^{13}C-NMR (DMSO-d_6) δ: 13.8 (5-CH_3), 13.8, 13.9, 22.0, 22.1, 24.4, 24.9, 28.3, 28.3, 28.4, 28.5, 31.0, 31.0, 33.6, 33.7, (21-OCO($\underline{C}H_2$)$_6\underline{C}H_3$), 16.2 (6-CH_3), 25.9 (16-CH_3), 27.4 (16-C), 30.1 (18-CH_3), 42.6 (15-C), 43.6 (10-C), 47.1 (8-C), 47.9 (4-C), 50.9 (9-C), 53.8 (17-C), 59.6 (3-C), 70.9 (7-C), 72.0 (18-C), 74.3 (21-C), 124.1 (19-C), 126.6 (4′-C), 127.1 (13-C), 128.0 (5-C), 128.4 (3′,5′-C), 128.5 (6-C), 129.4 (2′,6′-C), 134.3 (14-C), 137.3 (1′-C), 139.3 (20-C), 172.2 (7-acyl-CO), 172.4 (21-acyl-CO), 173.5 (1-C).

7-*O*-Acetylcytochalasin N (14) Cytochalasin N (**5**) (53 mg) was treated with Ac_2O (3.0 ml) and pyridine (2.0 ml) for 3 h at 40 °C and the precipitate formed by the addition of the reaction mixture to water was purified by the HPLC to give the 7-*O*-acetate (**14**) (48 mg), colorless powder, mp 140—142 °C (hexane–acetone). $[\alpha]_D^{23}$ +83.2° (c=0.30, MeOH). UV λ_{max}^{MeOH} nm (ε): 213 (10540). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1740, 1690, 1440, 1370, 1230, 1120, 960, 700. MS m/z: 535.2955 (M^+, Calcd for $C_{32}H_{41}NO_6$, 535.2936), 476, 444, 415, 372, 324, 306, 296, 281, 268, 251, 225, 210, 169, 157, 120, 105, 91, 43. ^{1}H-NMR (DMSO-d_6) δ: 0.935 (s, 6-CH_3), *ca.* 0.94 (16-CH_3), 1.173 (s, 18-CH_3), 1.381 (s, 5-CH_3), 1.430 (m, 17b-H), 1.556 (m, 15b-H), *ca.* 1.58 (m, 17a-H), 1.675 (m, 16-H), *ca.* 1.85 (m, 15a-H), 1.876 (s, 7-Ac), 2.266 (s, 21-Ac), 2.340 (s, 4-H), 2.565 (dd, J=9.9, 10.2 Hz, 8-H), 2.746 (dd, J=4.1, 12.4 Hz, 10a-H), 2.986 (dd, J=10.7, 12.4 Hz, 10b-H), 3.225 (dd, J=4.1, 10.7 Hz, 3-H), 5.101 (ddd, J=5.5, 11.5, 15.4 Hz, 14-H), *ca.* 5.14 (m, 7-H), 5.322 (d, J=16.8 Hz, 19-H), 5.426 (d, J=16.8, 20-H), 5.652 (dd, J= 10.2, 15.4 Hz, 13-H), 5.687 (s, 21-H), 7.21—7.34 (m, arom.-H), 8.292 (s, 2-NH). ^{13}C-NMR (DMSO-d_6) δ: 13.9 (5-CH_3), 16.4 (6-CH_3), 20.4 (–OCO–$\underline{C}H_3$), 20.5 (–OCO–$\underline{C}H_3$), 25.9 (16-CH_3), 27.4 (16-C), 30.2 (18-CH_3), 42.4 (15-C), 43.6 (10-C), 46.9 (8-C), 47.9 (4-C), 50.7 (9-C), 53.8 (17-C), 59.5 (3-C), 71.3 (7-C), 72.1 (18-C), 74.5 (21-C), 124.0 (19-C), 126.6 (4-C), 127.6 (5-C), 127.6 (13-C), 128.5 (3′,5′-C), 128.7 (6-C), 129.3 (2′,6′-C), 134.2 (14-C), 137.4 (1′-C), 139.4 (20-C), 169.8 (7-O$\underline{C}$O–CH_3), 170.1 (21-O$\underline{C}$O–CH_3), 173.6 (1-C).

18,21-*O*-Bis(*p*-dimethylaminobenzoyl)epoxycytochalasin J (15) A solution of *p*-dimethylaminobenzoic acid (1.0 g) in CH_2Cl_2 (5 ml) was treated with $SOCl_2$ (0.8 ml), and the reaction mixture was kept at 50 °C for 4 h under N_2 then evaporated *in vacuo*. Pyridine (1.0 ml) and 4-dimethylaminopyridine (trace) were added to epoxycytochalasin J (**2**) (170 mg) in CH_2Cl_2 (4 ml) and the solution was added to the chloride prepared above. The reaction mixture was stirred for 4 h at room temperature, then water was added. The whole was extracted with CH_2Cl_2 and the extract was purified by HPLC under the same condition as above to give the dibenzoate (**15**) (125 mg), colorless powder, mp 173—175 °C (hexane–acetone). $[\alpha]_D^{23}$ +93.3° (c=0.03, MeOH). UV λ_{max}^{MeOH} nm (ε): 206 (32740), 310 (46820). IR ν_{max}^{KBr} cm^{-1}: 3375, 2920, 1700, 1608, 1528, 1440, 1370, 1275, 1180, 1100, 1000, 960, 940, 825, 765, 700. MS (FAB(*m*-nitrobenzyl alcohol)) m/z: 745 (M^+). MS (EI) m/z: 580.3316 ($M^+ - C_9H_{10}NO_2$, calcd for 580.3301). ^{1}H-NMR (DMSO-d_6) δ: 0.156 (d, J=7.1 Hz, 5-CH_3), 1.074 (s, 6-CH_3), 1.084 (d, J=8.3 Hz, 16-CH_3), 1.292 (dq, J=5.5, 7.1 Hz, 5-H), 1.613 (s, 18-CH_3), *ca.* 1.67 (m, 16-H), *ca.* 1.72 (m, 17b-H), *ca.* 1.72 (m, 15b-H), 2.020 (d, J=5.5 Hz, 4-H), *ca.* 2.20 (m, 15a-H), *ca.* 2.20 (m, 17a-H), 2.571 (m, 8-H), 2.574 (m, 10a-H), 2.680 (d, J=5.5 Hz, 7-H), 2.949 (N-CH_3), 2.992 (N-CH_3), *ca.* 3.05 (m, 10b-H), 3.524 (m, 3-H), 5.363 (ddd, J= 5.2, 10.4, 15.4 Hz, 14-H), 5.564 (dd, J=2.5, 16.8 Hz, 20-H), 5.903 (s, 21-H), 5.968 (dd, J=2.2, 16.8 Hz, 19-H), 5.968 (dd, J=9.9, 15.4 Hz, 13-H), 6.65—7.88 (arom.-H), 8.406 (s, 2-NH). ^{13}C-NMR (DMSO-d_6) δ: 11.7 (5-CH_3), 19.1 (6-CH_3), 25.3 (16-CH_3), 26.9 (18-CH_3), 27.8 (16-C), 36.0 (5-C), 39.4 (N-CH_3), 39.6 (N-CH_3), 42.0 (15-C), 44.5 (8-C), 45.1 (10-C), 48.1 (4-C), 51.9 (9-C), 53.2 (3-C), 54.2 (17-C), 56.6 (6-C), 62.1 (7-C), 74.4 (21-C), 82.8 (18-C), 110.7, 111.1, 114.5, 116.9, 130.3, 130.7, 153.0, 153.5 (arom.-C in $(CH_3)_2NC_6H_4CO$), 125.3 (19-C), 126.4 (4′-C), 128.2 (3′,5′-C), 129.1 (13-C), 129.6 (2′,6′-C), 133.4 (14-C), 135.0 (20-C), 137.1 (1′-C), 164.8 (–OCOBz), 165.0 (–OCOBz), 174.1 (1-C).

Cell Morphology An established mouse cell line, C3H-2K, was generally used for determination of the effects of cytochalasins on cell morphology. C3H-2K cells were cultured in Dulbecco's modified Eagle's medium-10% fetal calf serum (FCS) containing 100 μg/ml kanamycin sulfate (Meiji Seika Kaisha Ltd., Tokyo). Confluent cultures of C3H-2K cells were trypsinized and plated in culture dishes as described,[2] and cultured at 37 °C for 4 h during which time the cells spread over the substrate to a considerable degree. Cytochalasins were added 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 gizzard actin antibody.[6] The method for staining was previously described.[2]

Lymphocyte Capping Splenic lymphocytes of Balb/c female mice (Charles River, Tokyo, Japan) were used for capping experiments. All the procedures were previously described in detail.[7]

Polymerization of Actin G-Actin was prepared from rabbit skeletal muscle acetone powder according to Spudich and Watt[8] and purified by Sephadex G-150 chromatography. *N*-(1-Pyrenyl)iodoacetamide was purchased from Molecular Probes, Junction City, OR. Pyrenyl actin was prepared as described by Cooper and Pollard[9] based on the findings of Kouyama and Mihashi,[10] and labeled actin was mixed with unlabeled actin to give a final concentration of 10% pyrenyl actin. Polymerization of G-actin (7.1 μM) was effected by adding 75 mM KCl in the presence of 0.2 mM adenosine triphosphate (ATP), 5 mM NaN_3, 0.1 mM $CaCl_2$ and 10 mM Tris–HCl buffer, pH 8.0 at 25 °C. Polymerization was usually monitored by measuring fluorescence intensity in a Hitachi 650-40 fluorescence spectrophotometer (excitation 365 nm; emission 407 nm). The rate of polymerization, given as arbitrary units of the increase in fluorescence intensity per min, was obtained from the continuous recording of the polymerization process. The rate was estimated by extrapolating the polymerization curve to zero time. However, when there was a lag in the initial stage of polymerization, the maximal rate was adopted. Cytochalasins were dissolved in dimethyl sulfoxide, and added to actin solution before the addition of salt. DMSO (5% (v/v)) was always added to the control.

Results

Alteration in Cell Morphology and Actin Distribution Most of the natural cytochalasins examined (**1**—**6**, **16**) exhibited similar biological effects on cultured cells to those of cytochalasin D and chaetoglobosin A.[2,3] As we have pointed out previously,[2] there is no strict correlation between the ability of cytochalasins to induce hairy structures containing actin and other effects of the same drugs. For instance, chaetoglobosin A is a weak inducer of hairy actin structures but a strong inhibitor of lymphocyte capping, whereas a positive correlation between these two effects was detected for the other cytochalasins listed in Table I. For scoring the effect on cell morphology, it was expressed as ++ when a drug at 2 μM was sufficient to induce hairy actin structures. When a drug was active at 20 μM but not at 2 μM, the effect was expressed as +. When no morphological alteration was induced at 20 μM, the effect was expressed as −.

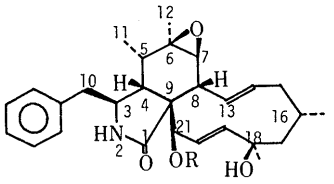
Inhibition of Lymphocyte Capping Mouse splenic lymphocytes were incubated with FITC-conjugated rabbit anti-mouse immunoglobulin (Ig) at 37 °C for 10 min. Generally, 85—95% of Ig-positive cells showed redistribution of surface Ig to patches followed by capping. When a cytochalasin was added at 20 μM to cells before incubation with FITC-rabbit anti-mouse Ig, the percentage of cells showing capping was reduced (Table I, column b). Natural cytochalasins (**1**—**6**, **10**, **16**) except cytochalasins P, Q and R (**7**—**9**) exhibited relatively strong inhibitions (50—80%), while synthetic derivatives (**11**—**15**) showed weaker inhibitions. The novel cytochalasins having a 6,7-glycol group (**7**—**9**) showed little or no inhibition.

Inhibition of Actin Polymerization In the previous papers,[2,3] a good correlation between the effects at the cellular level (inhibition of capping) and the *in vitro* effects

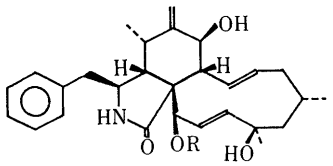
TABLE I. The Effects of New Cytochalasins from *Phomopsis* sp. and Their Derivatives on Cellular Structures and Actin Polymerization

Sample No.	(a) Formation of hairy structures	(b) Inhibition of capping (%)	(c) Rate of actin polymerization[a] (%)	(c) Inhibition of actin polymerization[b] (%)
Epoxycytochalasin H (**1**)	++	79	17	54
Epoxycytochalasin J (**2**)	++	55	17	37
Cytochalasin H (**3**)	++	57	18	49
Cytochalasin J (**4**)	++	58	21	45
Cytochalasin N (**5**)	++	72	14	48
Cytochalasin O (**6**)	++	50	20	43
Cytochalasin P (**7**)	−	7	100	0
Cytochalasin Q (**8**)	−	0	100	0
Cytochalasin R (**9**)	−	38	100	0
Cytochalasin S (**10**)	+	60	100	0
21-*O*-Octanoylepoxycytochalasin J (**11**)	+	48	11	46
21-*O*-Octanoylcytochalasin O (**12**)	+	49	22	31
7,21-*O*-Dioctanoylcytochalasin O (**13**)	−	25	100	4
7-*O*-Acetylcytochalasin N (**14**)	−	31	100	2
18,21-*O*-Bis(*p*-dimethylaminobenzoyl) epoxycytochalasin J (**15**)	−	22	100	3
Pyrichalasin H (**16**)	++	62		
Cytochalasin D	++	70	11	36
Chaetoglobosin A	±	89		

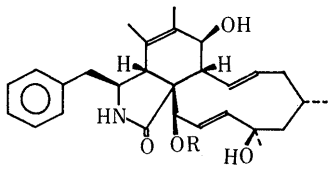
a) Relative rate of increase in fluorescence intensity per min, given as arbitrary units. *b*) Relative value of fluorescence intensity as an indicator of extent of polymerization.



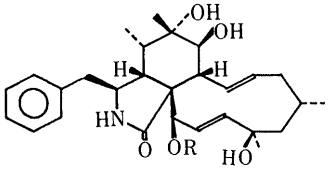
1 : R=Ac epoxycytochalasin H
2 : R=H epoxycytochalasin J



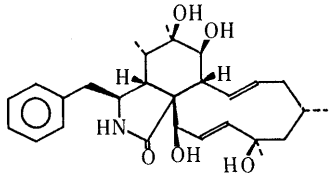
3 : R=Ac cytochalasin H
4 : R=H cytochalasin J



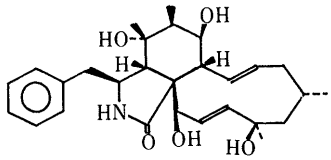
5 : R=Ac cytochalasin N
6 : R=H cytochalasin O



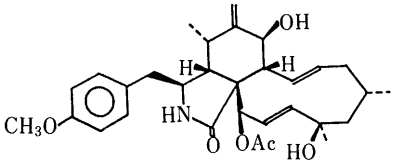
7 : R=Ac cytochalasin P
8 : R=H cytochalasin Q



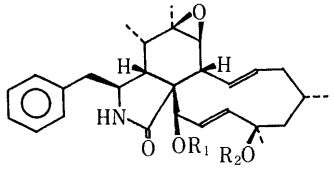
9 : cytochalasin R



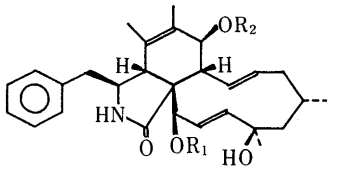
10 : cytochalasin S



16 : pyrichalasin H



11 : R_1=$OC(CH_2)_6CH_3$, R_2=H
15 : R_1=R_2=$OCC_6H_4N(CH_3)_2$



12 : R_1=$OC(CH_2)_6CH_3$, R_2=H
13 : R_1=R_2=$OC(CH_2)_6CH_3$
14 : R_1=R_2=Ac

Chart 1

(inhibition of actin filament elongation determined by viscometry) was reported. In the present study, the inhibitory action of the cytochalasin derivatives on actin polymerization was tested using pyrenyl actin.[11] Two parameters, *i.e.* initial rate of actin polymerization and the extent of polymerization at equilibrium, were determined.

As summarized in Table I, column (c), the natural products (**1**—**6**) caused marked decreases in both the rate and extent of actin polymerization. Interestingly, the inhibition was more remarkable in the initial rate of polymerization (80—86% inhibition) than in the final extent of polymerization (37—54% inhibition). On the other hand, the synthetic derivatives (**13**—**15**) did not show any inhibition. The novel glycol-type cytochalasins (**7**—**10**) were also not effective at all. It is noteworthy that, in the case of three compounds, the *in vitro* effects did not show a good correlation with the cellular effects: two synthetic derivatives (**11**, **12**) had a strong *in vitro* effects though their

cellular effects were not so marked, while cytochalasin S (**10**) did not exhibit any *in vitro* activity though the compound showed activities at the cellular level.

Discussion

Fifty-four compounds belonging to the category of cytochalasins have so far been isolated from diverse fungal sources.[4,5,12-14] The common structural feature of the group is expressed by a perhydroisoindol-1-one moiety bearing a benzyl (cytochalasins), *p*-methoxybenzyl (pyrichalasin (**16**)), (indol-3-yl)methyl (chaetoglobosins), or 2-methylpropyl (aspochalasins) group at the C_3 position and an 11-, 13- or 14-membered carbocyclic (or oxygen-containing) ring connecting the C_8 and C_9 positions. The variety of the structures is due to the size of the macrocyclic ring which has several functions beyond C_{17}, different substituents at the C_3 and the oxygen functions and a double bond at C_5–C_7.[13,14]

Our previous work[2] revealed that the most of the effects caused by cytochalasins involve the interaction of the drugs with the target protein, actin. The structure–activity relationships of the cytochalasins so far known can be summarized as follows. Compounds with aromatic substituents at C_{10} (phenyl or indolyl; cytochalasins and chaetoglobosins) show the same magnitude of effects, while the isopropyl compounds (aspochalasins) are relatively ineffective. The perhydroisoindol-3-one nucleus is a most important factor in the activities, but the position of the double bond and the presence or absence of the oxygen function (6-ene, 6,7-epoxide, 6(12)-en-7-ol, or 5-en-7-ol) do not influence the effects. The macrocyclic ring starting from C_8 and running to C_9 is essential but definite functional groups or carbon numbers of the ring over C_{17} appeared to be unnecessary for the effects.[3] It was also suggested that the physical properties of the compounds such as lipophilicity, influence their relative effectiveness.[3]

Sixteen compounds examined in this study belong to the class of 10-phenyl-[11]cytochalasans.[13,14] The above generalizations were mostly applicable to the compounds in this study. Epoxycytochalasins H and J and cytochalasins H, J, N and O (**1**—**6**), in which the 6,7-epoxide, 6(12)-en-7-ol or 5-en-7-ol part of the molecules and the presence and absence of an acetyl group at C_{21}-OH are different but other parts are identical, showed nearly the same magnitudes of the activities. Pyrichalasin H (**16**),[5] having a *p*-methoxyphenyl group instead of the phenyl group of cytochalasin H (**3**), showed the same activity as **3**, indicating that the methoxyl group does not influence the activities.

The most striking result is the inactivity or relatively weak effects exhibited by cytochalasins P—S (**7**—**10**), novel 5,7- or 6,7-diol type compounds, especially *in vitro*. The ^{1}H-NMR examinations of these compounds[4] suggested that boat or half-boat conformations of the same type were adopted as in the case of other cytochalasins. Although the conformations of these compounds in biological systems are uncertain, it was suggested that the presence of an additional hydroxyl group at the C_5 or C_6 position besides the C_7-hydroxyl group has an unfavorable effect on the binding site.

The idea for the preparation of the derivatives (**11**—**15**) was based on the expectation that a change of the lipophilicity of the compounds could be produced by the introduction of substituents at C_{21}, in the domain assumed not to be particularly influential on the biological effects.[3] Compounds **13** and **14**, in which the active 7-hydroxyl group is acylated, as well as the 18,21-*O*-dibenzoyl derivative (**15**), in which the 6,7-epoxide function is retained, showed weaker effects, especially at the cellular level, than the original compound, while the 21-*O*-octanoyl derivatives (**11**, **12**) showed nearly the same activities as the original compounds (**2**, **6**). ^{1}H-NMR examination (see data in Materials and Methods) indicated that the chair-like (zig-zag) conformation of the eleven-membered ring is retained in these compounds.[15,16] Thus, as a whole, the lipophilicity itself is not a critical factor for the relative effectiveness.

Recently Nukina[5] reported the inhibition of the growth of rice seedlings by pyrichalasin H (**16**). Epoxycytochalasins H and J and cytochalasins H, J, N and O exhibited the same effects at 10 ppm but P (**7**) and Q (**8**) did not show the effect at concentration of up to 20 ppm.[17]

Acknowledgements The authors thank Dr. M. Nukina, Yamagata University, for his generous gift of the sample of pyrichalasin H and the data on the effects on rice seedlings.

Added in Proof (October 30, 1989) Although a preliminary communication of our work on cytochalasins from *Phomopsis* sp. (T. Tomioka, Y. Izawa, K. Koyama and S. Natori, *Chem. Pharm. Bull.*, **35**, 902 (1987)) appeared first, five other different cytochalasins from *Hypoxylon terricola*, named cytochalasins N, O, P, Q and R, were reported[12] a little earlier than our full paper on cytochalasins N—S from *Phomopsis* sp.[4] By the negotiation with the British workers, we have now agreed to distinguish the two series by using the subscripts pho and hyp indicating the source of microorganisms, following the proposal made in the case of cytochalasin K (A. H. Kapadi and Sukh Dev, "Recent Advances in Cytochalasans," ed. by G. S. Pendswe, Indian Drugs Research Association, Pune, 1986, p. 30). Thus cytochalasins N, O, P, Q and R in this paper will hereafter be renamed as cytochalasins N_{pho}, O_{pho}, P_{pho}, Q_{pho} and R_{pho}, respectively.

References and Notes

1) In the chemical literature, cytochalasins are now accepted as the trivial names of the 10-phenyl compounds in the group and the group as a whole should be called cytochalasans. However, the commonly used term 'cytochalasins' will be used to cover all the compounds discussed in this paper.
2) I. Yahara, F. Harada, S. Sekita, K. Yoshihira and S. Natori, *J. Cell. Biol.*, **92**, 69 (1982)
3) S. Sekita, K. Yoshihira, S. Natori, F. Harada, K. Iida and I. Yahara, *J. Pharmacobio-Dyn.*, **8** 906 (1985).
4) Y. Izawa, T. Hirose, T. Shimizu, K. Koyama and S. Natori, *Tetrahedron*, **45**, 2323 (1989).
5) M. Nukina, *Agric. Biol. Chem.*, **51**, 2625 (1987).
6) K. Iida and I. Yahara, *Exp. Cell Res.*, **164**, 492 (1986).
7) I. Yahara and G. M. Edelman, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 608 (1972).
8) J. A. Spudich and S. Watt, *J. Biol. Chem.*, **246**, 4866 (1971).
9) J. A. Cooper and T. D. Pollard, *Methods Enzymol.*, **85**, 182 (1982).
10) T. Kouyama and K. Mihashi, *Eur. J. Biochem.*, **114**, 33 (1981).
11) K. Maruyama, M. Oosawa, A. Tashiro, T. Suzuki, M. Tanikawa, M. Kikuchi, S. Sekita and S. Natori, *Biochem. Biophys. Acta*, **874**, 137 (1986).
12) R. L. Edwards, D. L. Maitland and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, **1989**, 57.
13) S. Natori, K. Iida and I. Yahara, *Tanpakushitsu Kakusan Koso*, **28**, 789 (1983).
14) S. Notori, *Yakugaku Zasshi*, **103**, 1009 (1983).
15) S. Sekita, K. Yoshihira, S. Natori and H. Kuwano, *Chem. Pharm. Bull.*, **30**, 1629 (1982).
16) S. Sekita, K. Yoshihira and S. Natori, *Chem. Pharm. Bull.*, **31**, 490 (1983).
17) Dr. M. Nukina, private communication.