Communications to the Editor

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THREE NEW 10-PHENYL-[11]CYTOCHALASANS, CYTOCHALASINS N, O, AND P FROM PHOMOPSIS SP.

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From Phomopsis sp.(68-GO-164) three new cytochalasans named cytochalasins N, O, and P were isolated, besides the four known cytochalasans, cytochalasins H and J and epoxycytochalasins H and J. The structures of the new compounds were determined by spectral analysis, especially by NMR, and by correlation reactions with known compounds.

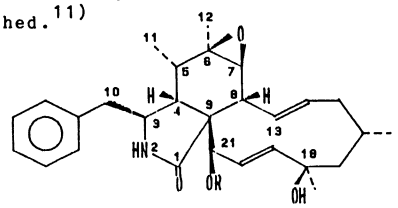
KEYWORDS——cytochalasan; cytochalasin; Phomopsis sp.; ^{1}H-NMR; ^{13}C-NMR; cytotoxicity

In the course of our studies on mycotoxin-production by food-borne fungi collected in Japan, Chaetomium spp. were found to produce eight novel cytochalasans with an indol-3-yl group.[2] They were designated chaetoglobosins A-G and J. Twelve strains of the molds exhibiting cytotoxicity to HeLa cells with polynuclear cell formation[3] were investigated with regard to the production of metabolites that affect microfilaments (actin) and microtubules (tubulin). Nine of the strains showed no such effects, probably due to the loss of metabolic activity during storage. But Phomopsis sp., Diaporthe phaseolorum, and Pithomyces sacchari exhibited the same type of the cytotoxicity.[4]

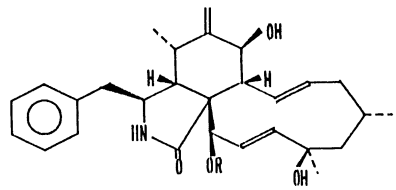
The metabolites of Phomopsis sp. (68-GO-164) were investigated in detail. The culture conditions were examined by monitoring cytotoxicity. Culturing on wheat for 20 days at 26°C was found to give a good yield of the metabolites. The moldy wheat thus obtained was extracted with dichloromethane and the extracts were chromatographed on silica gel (hexane-acetone). The toxic fractions were further separated by HPLC using Nucleosil 50-5 (hexane-acetone). After spraying with 50% methanolic sulfuric acid and heating, the fractions containing cytochalasans were detected on TLC as bright yellow fluorescent spots under UV light. Nine compounds were thus separated and characterized. First two compounds, which were not cytotoxic after purification, were identified as (3S,4S)cis- and trans-4-hydroxymellein.[5] The other seven compounds (1 - 7) were proved to belong to the 10-phenyl-[11]cytochalasans, a group of cytotoxic mycotoxins that bind specifically with actin.

According to elemental analyses and mass spectrometry, three of these compounds(1 - 3) had the same molecular formula $C_{30}H_{39}NO_5$, three others (4 - 6) had the formula $C_{28}H_{37}NO_4$, and the seventh (7) had $C_{30}H_{41}NO_6$. Nuclear magnetic resonance (NMR) data revealed that the three (1 - 3) are the acetyl derivatives of the other three (4 - 6) respectively. Detailed ^{1}H- and ^{13}C-NMR studies of the metabolites indicated that the compounds (1, 2, 4, and 5) were epoxycytochalasin H, cytochalasin H (kodocytochalasin-1, paspalin P1), epoxycytochalasin J (epoxydeacetylcytochalasin H), and cytochalasin J (kodocytochalasin-2, paspalin P2, deacetylcytochalasin H). These had been previously isolated from Phomopsis spp.,[6-9] and their identities were established by direct comparison with authentic samples.

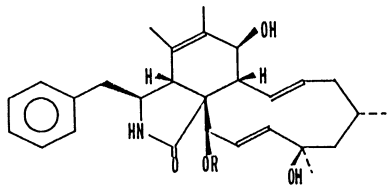
The other three compounds were new: cytochalasin N (3), colorless powder, mp 253-254°C (acetone), $[\alpha]_D$ + 85.4° (MeOH), λ_{max}^{MeOH} 208 nm (ε 19800), ν_{max}^{KBr} cm^{-1}: 3400, 2725, 1690, 1470, 1235, 1150, 960, 700; cytochalasin O (6), colorless needles, mp 187-188 °C (hexane-acetone), $[\alpha]_D$ +59.6° (MeOH), λ_{max}^{MeOH}: 206 nm (ε 20800), ν_{max}^{KBr} cm^{-1}: 3425, 1670, 1250, 1140, 960, 700; and cytochalasin P (7), mp 117-118°C ($CHCl_3$), $[\alpha]_D$ -116° (MeOH), λ_{max}^{MeOH} 208 nm (ε 15200), ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1680, 1370, 1230, 960, 700. The NMR spectra of 3 and 6 (Table I) indicated that, excepting the cyclohexane part of the molecule, they have the same structures as 1 and 2 and 4 and 5 respectively, and they are 5(6)-en-7β-ol derivatives like cytochalasin C[10] and chaetoglobosin B.[2] To confirm the structures, the correlation reactions shown in Chart 1 were performed and the structures of cytochalasins N (3) and O (6) were established.[11]



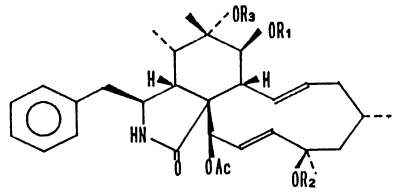
(1) R=Ac epoxycytochalasin H
(4) R=H epoxycytochalasin J



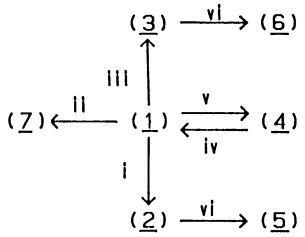
(2) R=Ac cytochalasin H
(5) R=H cytochalasin J



(3) R=Ac cytochalasin N
(6) R=H cytochalasin O



(7) R_1=R_2=R_3=H cytochalasin P
(8) R_1=benzoyl R_2=R_3=H
(9) R_1=R_2=benzoyl R_3=H
(10) R_1=R_2=R_3=benzoyl



Reagents : i) Al[OCH(CH3)2]3 / toluene
ii) CF3CO2H / CH3CN
iii) H2SO4 / DMSO
iv) Ac2O / pyridine
v) NaOH / t-BuOH
vi) 5% KOH

Chart I

Comparison of the physical data and the molecular formulae indicated that the seventh congener (**7**) corresponds to the hydrate of **1**, **2**, and **3**. The ^{1}H- and ^{13}C-NMR data of **7** were precisely analyzed using COSY, C-H COSY, and DEPT. The results (Table I) clearly indicated the presence of a tert-hydroxyl and a methyl group at C_6 besides secondary hydroxyl group at C_7. Treatment of epoxycytochalasin H (**1**) with trifluoroacetic acid in acetonitrile[12] gave **7** in a 30% yield, besides **2** and **3**. Thus the compound (**7**) was proved to be a 6,7-diol-type compound, which had not been found previously among the forty-three cytochalasans so far characterized.[13]

Table I ^{1}H- and ^{13}C-NMR Data of Cytochalasins N (3), O (6), and P (7) (in DMSO-d_6 at 400 MHz for ^{1}H and 100 MHz for ^{13}C)

	Cytochalasin N (3)	Cytochalasin O (6)	Cytochalasin P (7)
		^{1}H	
2	8.15 (s)	7.83 (s)	7.92 (s)
3	3.17 (ddd, 4.3, 10.5, --)	3.12 (ddd, 5.0, 9.8, --)	4.02 (ddd, 4.6, 5.4, 5.0)
4	2.27 (m)	2.85 (m)	1.75 (dd, 5.0, 5.1)
5	-----	-----	1.63 (dq, 5.1, 7.0)
7	3.60 (m)	3.57 (m)	3.27 (dd, 5.9, 11.4)
8	2.36 (dd, 10.0, 10.0)	2.36 (dd, 10.1, 10.1)	2.46 (dd, 11.4, 10.2)
10	2.96 (dd, 12.8, 4.3) 2.72 (dd, 12.8, 10.5)	2.92 (dd, 12.9, 5.0) 2.70 (dd, 12.9, 9.8)	2.57 (dd, 13.8, 5.4) 2.78 (dd, 13.8, 4.6)
11	1.51 (s)	1.51 (s)	0.81 (d, 7.0)
12	0.87 (s)	0.95 (s)	0.98 (s)
21	5.66 (dd, 1.8, 2.0)	5.27 (s)	4.77 (dd, 2.4, 2.1)
21-Ac	2.25 (s)	-----	2.01 (s)
		^{13}C	
1	174.5 (s)	176.6 (s)	174.3 (s)
3	49.1 (d)	48.5 (d)	52.6 (d)
4	60.0 (d)	59.8 (d)	48.8 (d)
5	125.5 (s)	126.4 (s)	37.8 (d)
6	133.2 (s)	132.4 (s)	75.0 (d)
7	68.3 (d)	68.6 (d)	75.5 (d)
8	48.5 (d)	48.0 (d)	45.4 (d)
9	51.4 (s)	53.1 (s)	52.9 (s)
10	43.3 (t)	43.4 (t)	42.9 (t)
11	14.4 (q)	14.3 (q)	12.8 (q)
12	16.5 (q)	16.6 (q)	22.4 (q)
21	75.1 (d)	73.4 (d)	77.6 (d)
21-Ac	20.4 (q) 170.4 (s)	----- -----	20.5 (q) 169.2 (s)

The stereochemistry of the glycol part was established as follows: The coupling constant of the C_7 and C_8 protons (11.4 Hz) showed the β-configuration of the C_7-hydroxyl group. Although the formation of **7** from **1** by the cleavage of the epoxide indicated the trans configuration of the glycol,[14] further experiments to confirm the configuration were carried out by two methods. First, the relative stereochemistry of the C_6-methyl group and protons at C_5 and C_8 were confirmed by NOE difference spectra. Second, the dibenzoate chirality rule was applied as follows: Treatment of cytochalasin P (**7**) with benzoyl chloride in pyridine gave 7-monobenzoate (**8**), $[\theta]$ -1.93×10^3(280 nm), 7,18-dibenzoate (**9**), $[\theta]$ -3.69×10^3(277 nm), and 6,7,18-tribenzoate (**10**), $[\theta]$ $+3.65\times10^4$(270 nm), according to the reaction conditions. Comparing the CD spectra of these compounds showed the positive effect of the 6,7-dibenzoate, indicating the α-configuration of the C_6-hydroxyl. Thus the stereochemistry of the glycol part was established.

The ED_{50} values of the cytochalasins (**1** - **6**) to HeLa cells were in the range of 0.32-3.2 μg/ml. However, cytohalasin P (**7**), with an additional hydroxyl group at the 6-position, was much less cytotoxic. The structure-activity relationship of these compounds to cell phenomena and actin in vitro[15] is under investigation.

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