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SIX NEW 10-PHENYL-[11]CYTOCHALASANS, CYTOCHALASINS N - S FROM *Phomopsis* SP.

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Abstract: From *Phomopsis* sp. (68-GO-164) six new cytochalasans named cytochalasins N, O, P, Q, R and S were isolated, together with the four known compounds, epoxycytochalasins H and J and cytochalasins H and J. The structures (**5** - **10**) of the new compounds as 10-phenyl-[11]cytochalasans were determined from spectral data, especially ^{1}H and ^{13}C NMR, and by chemical correlation with known compounds. Cytochalasins P, Q, R and S (**7** - **10**) have novel diol-type structures in the cyclohexane part of the molecules.

The cytochalasans are a group of fungal secondary metabolites, which inhibit a variety of cellular movements including cell division and motility, and cause changes in cell shape.[1,2] About forty naturally occurring cytochalasans are so far known.[3] Most of the effects caused by cytochalasans can be attributed to the interaction between these drugs and the common target protein, actin.[2,3]

In the course of our studies on mycotoxin production by food-borne fungi, eight novel indolyl-[13]cytochalasans, designated chaetoglobosins A - G and J, were isolated from *Chaetomium* spp. and their structures were determined.[2] Reinvestigation of the molds exhibiting cytotoxicity to HeLa cells with polynuclear cell formation [mainly caused by metabolites that affect microfilaments (actin) and microtubules (tubulin)] revealed the production of such metabolites by *Phomopsis* sp. (68-GO-164), *Diaporthe phaseolorum*, and *Pithomyces sacchari*.[4] This paper concerns the characterization of ten 10-phenyl-[11]cytochalasans including six new compounds from *Phomopsis* sp.[5]

The dichloromethane extract of the culture on wheat at 26°C for 20 days of *Phomopsis* sp. (68-GO-164 strain)[6] was separated by silica gel chromatography and HPLC using Nucleosil 50-5. The fractions containing cytochalasans were detected as fluorescent spots under an UV light on TLC plates after spraying 50% sulfuric acid and heating[7] and by bioassay of cytotoxicity.[4] Besides (3S,4S)-*cis*- and *trans*-4-hydroxymellein,[8] ten cytochalasans were isolated. They showed characteristic physical properties of 10-phenylcytochalasans such as the base peak at 91 *m/z* in the mass spectra (tropylium ion), and absorptions at 1680, 1600 and 1450 cm^{-1} in the IR spectra. From the molecular formulae determined by high resolution MS and the ^{1}H and ^{13}C NMR data (Tables I

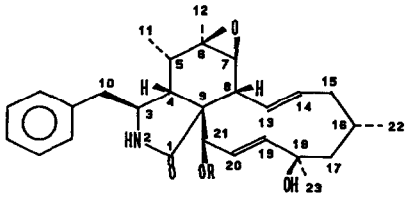
and II), four of these compounds were suggested to be epoxycytochalasin H (**1**), the major metabolite, epoxycytochalasin J (epoxydeacetylcytochalasin H) (**2**), cytochalasin H (kodocytochalasin-1, paspalin P1) (**3**), and cytochalasin J (kodo-cytochalasin-2, paspalin P2, deacetylcytochalasin H) (**4**), previously isolated from *Phomopsis* spp.[7,9-12] and their identities were established by direct comparison with authentic samples. Correlation reactions of these compounds were carried out as shown in Chart 1 for further confirmation of the structures and the ambiguities in the NMR assignments in the literature[9,12] were removed by precise decoupling experiments.

The other six compounds were new compounds and were named cytochalasins N, O, P, Q, R and S. Molecular formulae were established by high resolution MS and elemental analyses as follows N (**5**), $C_{30}H_{39}NO_5$, O (**6**), $C_{28}H_{37}NO_4$, P (**7**), $C_{30}H_{41}NO_6$, and Q (**8**), R (**9**) and S (**10**), $C_{28}H_{39}NO_5$. The spectral data also suggested that N (**5**) and P (**7**) are monoacetates of O (**6**) and Q (**8**) respectively.

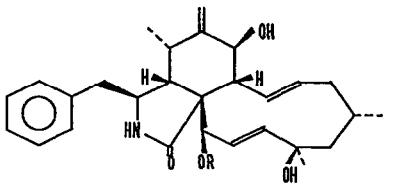
The spectral data, especially ^1H and ^{13}C NMR (Tables I and II), of N (**5**) and O (**6**) indicated that, excepting the cyclohexane part of the molecules, they have the same structures as epoxy H (**1**) and F (**3**) and epoxy J (**2**) and J (**4**), respectively. Two allyl methyl groups and one secondary alcohol group in the six membered ring suggested the 5(6)-en-7-ol structure in the cyclohexane part of the cytochalasans as in cytochalasin C[13] and chaetoglobosin B.[14] The stereochemistry of the alcohol group was suggested to be β by the coupling constant (J_{7-8} 10 Hz). To confirm the structures, epoxycytochalasin H (**1**) was treated with sulfuric acid in DMSO to give N (**5**) in a good yield, while hydrolysis of N (**5**) with alkali gave O (**6**). Thus the structures were established.

The physical data and the molecular formulae of cytochalasins P (**7**) and Q (**8**) indicated that P (**7**) is the monoacetate of Q (**8**), and they correspond to the hydrates of **1**, **3**, and **5** and **2**, **4**, and **6**, respectively, the structural difference exists in the cyclohexane moiety. Since the structure in this part of the molecules was assumed to be different from those of the cytochalasans so far known, detailed ^1H and ^{13}C NMR analyses using COSY, C-H COSY, and DEPT were performed. As the results, three bond sequences, from C_{10} phenyl to C_5 methyl, from C_7 sec-hydroxyl to C_{17} methylene, and from C_{19} methine to C_{21} methine, and the presence of two quaternary carbons carrying a tert-hydroxyl and a methyl group at C_{18} and, probably, at C_6 were deduced. Thus a new type of structure of the cyclohexane part, 6,7-glycol, was proposed. To confirm the structures, P (**7**) was hydrolyzed with sodium hydroxide in acetonitrile to give Q (**8**) and epoxy H (**1**) was treated with trifluoroacetic acid in acetonitrile[15] to give P (**7**) in a 30% yield, besides H (**3**) and N (**5**).

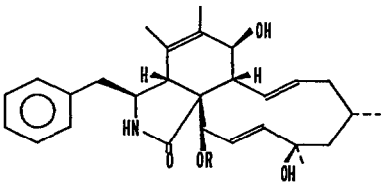
The stereochemistry (Chart 2) of the glycol part of the molecule was established as follows: The coupling constant (J_{7-8} 11.4 Hz) showed the β configuration of the C_7-hydroxyl group. The formation of P (**7**) from epoxy H (**1**) by the cleavage of the epoxide ring indicated the trans-glycol configuration.[16] Since positive NOE was observed in NOE difference spectra of the 7-O-acetate (**7a**) [prepared from P (**7**)] at the C_5- and C_8-protons on irradiation of C_6-methyl protons, the methyl group was suggested to be β. Furthermore, the dibenzoate chirality rule was applied to the 7-mono- (**7b**), 7,18-di- (**7c**), and 6,7,18-tri-benzoates (**7d**) of P (**7**) (the structures were confirmed



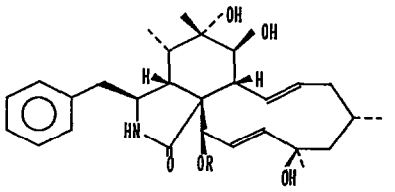
(1) R=Ac epoxycytochalasin H
(2) R=H epoxycytochalasin J



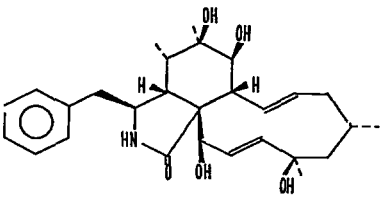
(3) R=Ac cytochalasin H
(4) R=H cytochalasin J



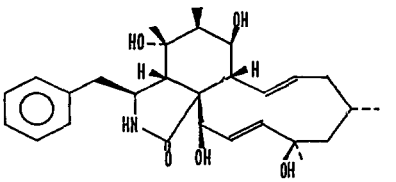
(5) R=Ac cytochalasin N
(6) R=H cytochalasin O



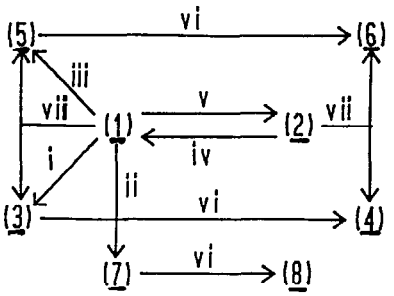
(7) R=Ac cytochalasin P
(8) R=H cytochalasin Q



(9) cytochalasin R



(10) cytochalasin S



Reagent
i) $Al_3[OCH(CH_3)_2]_3$ / toluene
ii) CF_3CO_2H / CH_3CN
iii) H_2SO_4 / DMSO
iv) Ac_2O / pyridine
v) NaOH / t-BuOH
vi) KOH / MeOH(CH_3CN)
vii) 90% AcOH

Chart 1 Structures and Correlation Reactions of the Cytochalasans from *Phomopsis* sp. (68-GO-164)

Table I ^{1}H NMR Data of Cytochalasins

chemical shifts (ppm) in DMSO-d_6 at 400 MHz

proton at	epoxy-cytochalasin H (1)	epoxy-cytochalasin J (2)	cytochalasin H (3)	cytochalasin J (4)	cytochalasin N (5)	cytochalasin O (6)	cytochalasin P (7)	cytochalasin Q (8)	cytochalasin R (9)	cytochalasin S (10)
2	8.308 (s)	7.965 (s)	8.012 (s)	7 694 (s)	8 150 (s)	7.827 (s)	7.925 (s)	7.651 (s)	7.814 (s)	7.833 (s)
3	3.520 (m)	3.508 (m)	3 096 (m)	3.093 (m)	3.171 (m)	3 120 (m)	4.018 (ddd)	4.039 (ddd)	3.425 (m)	3.557 (ddd)
4	1.931 (m)	2 463 (m)	1 982 (m)	2.546 (m)	ca.2 27	ca.2.85	1.745 (dd)	2.068 (dd)	2.245 (dd)	2 076 (d)
5	1 461 (m)	1 464 (m)	2.466 (m)	2.466 (m)	-	-	1.630 (m)	1.785 (dq)	1.985 (dq)	-
6	-	-	-	-	-	-	-	-	-	ca.1.53
7	2.599 (d)	2.543 (d)	3.630 (dd)	ca.3.55	3.598 (m)	3.566 (m)	3.267 (dd)	3.285 (dd)	2.905 (d)	2.851 (m)
8	2 423 (dd)	2.416 (dd)	2.743	2.713	2.355	2 357	2.461 (dd)	ca.2.50	2.735 (dd)	3.043 (dd)
10a	2.953 (dd)	2.856 (dd)	2.831 (dd)	ca.2.71	2.964 (dd)	2.915 (dd)	2.566 (dd)	2.528 (dd)	ca.2 66	2.650 (dd)
10b	ca.3 40	ca.3 40	2 547 (dd)	2 634 (dd)	2 719 (dd)	2 703 (dd)	2.778 (dd)	2.853 (dd)		ca.2.90
11	0 312 (d)	0.479 (d)	0 375 (d)	0.569 (d)	1.507 (s)	1 507 (s)	0.805 (d)	0.932 (d)	0.681 (d)	1.130 (d)
12a	1 076 (s)	1.092 (s)	4.799 (s)	4.800 (s)	0.873 (s)	0 951 (s)	0 982 (s)	0.969 (s)	1.007 (s)	1.030 (d)
12b			5.039 (s)	5 043 (s)						
13	5 811 (dd)	5.767 (dd)	5.535 (dd)	5 471 (dd)	5.720 (dd)	5.712 (dd)	5.376 (dd)	5.323 (dd)	5.372 (dd)	5.393 (dd)
14	5.159 (ddd)	5.102 (ddd)	5 081 (ddd)	5.024 (ddd)	5.065 (ddd)	5 036 (ddd)	4.905 (ddd)	4.881 (ddd)	4.922 (ddd)	4.889 (ddd)
15a	1.910 (m)	1 879 (m)	1.897 (m)	1 852 (m)	1.906 (m)	1.870 (m)	ca 1.58	1.536 (ddd)	1 536 (ddd)	ca 1 53
15b	ca.1.63	ca 1.57	ca.1.60	ca.1.56	ca.1 65	ca 1.62	1.884 (ddd)	1 850 (ddd)	1.848 (ddd)	1.866 (ddd)
16	ca 1.69	ca.1 71	ca.1.67	ca.1.68	ca 1 68	ca.1 71	1.685 (m)	ca.1.68	ca.1.68	1.720 (m)
17a	1.600 (dd)	1.629 (dd)	1.609 (dd)	1.622 (dd)	1 617 (dd)	1.653 (dd)	1.352 (dd)	1.325 (dd)	1.340 (dd)	1.330 (dd)
17b	1.420 (dd)	1.397 (dd)	1.401 (dd)	1.370 (dd)	1 420 (dd)	1 402 (dd)	ca.1.60	1.625 (dd)	1.617 (dd)	1.592 (dd)
19	5.660 (d)	5.753 (d)	5.669 (dd)	5.758 (dd)	5 676 (dd)	5 766 (d)	5.746 (dd)	5.511 (dd)	5.585 (dd)	5.578 (dd)
20	5 399 (d)	5.692 (d)	5 371 (dd)	5 650 (dd)	5 405 (dd)	5 700 (d)	5.247 (dd)	5.883 (dd)	5.825 (dd)	5.866 (dd)
21	5.533 (s)	5.142 (d)	5.297 (s)	5.095 (d)	5 664 (s)	5.269 (d)	4.774 (dd)	3.313 (ddd)	3.480 (dd)	3.243 (m)
16-CH_3	1.004 (d)	0.957 (d)	0.953 (d)	0.951 (d)	0 959 (d)	0.963 (d)	0.929 (d)	0 929 (d)	0.930 (d)	0.920 (d)
18-CH_3	1 172 (s)	1.180 (s)	1.146 (s)	1 144 (s)	1.176 (s)	1 193 (s)	1.088 (s)	1.100 (s)	1.120 (s)	1.102 (s)
2'6'	7.184 (d)	7.234 (d)	7.149 (d)	7.215 (d)	7 200 (d)	7.289 (d)	7 124 (d)	7.176 (d)	ca.7.21	ca.7.21
3'5'	7.223 (dd)	7.296 (dd)	7.290 (dd)	7.289 (dd)	7.319 (dd)	7.321 (dd)	7.271 (dd)	7.276 (dd)	7.290 (dd)	7.290 (dd)
4'	7.193 (t)	7 263 (t)	7.217 (t)	7.212 (t)	7.242 (t)	7.241 (t)	7.195 (dd)	7.184 (dd)	ca.7.21	ca.7.21
21-OAc	2.203 (s)	-	2.236 (s)	-	2.250 (s)	-	2.010 (s)	-	-	-
21-OH	-	4.007 (d)	-	4.327 (d)	-	4.308 (d)	-	4.751 (d)		5.414 (d)
5-OH	-	-	-	-	-	-	-	-	-	
6-OH	-	-	-	-	-	-	4.312 (s)	4.151 (s)		-
7-OH	-	-	4 508 (d)	3 711 (d)	4 183 (d)	3.877 (d)	4.018 (d)	3.746 (d)		3.877 (d)
18-OH	4.415 (s)	4.241 (s)	4.395 (s)	4.250 (s)	4.448 (s)	4.276 (s)	4.271 (s)	4.142 (s)		

Proton-proton coupling constants (Hz)

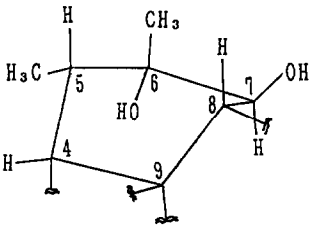
	epoxy-cytochalasin H (1)	epoxy-cytochalasin J (2)	cytochalasin H (3)	cytochalasin J (4)	cytochalasin N (5)	cytochalasin O (6)	cytochalasin P (7)	cytochalasin Q (8)	cytochalasin R (9)	cytochalasin S (10)
$J_{3\ 4}$		1.9					5.0	5.0	5.0	4.7
$J_{3\ 10a}$	4.0	4.4	4.9		4.3	5.0	5.4	3.8		5.2
$J_{3\ 10b}$	9.3		8.6	7.0	10.5	9.8	4.6	5.7		
$J_{10a\ b}$	12.6	13.0	12.8	13.1	12.8	12.9	13.8	13.7		13.8
$J_{4\ 5}$	5.5	5.5			-	-	5.1	5.0	5.0	-
$J_{5\ 11}$	7.1	7 3	6.4	6.7	-	-	7.0	7.1	7.2	-
$J_{6\ 7}$	-	-	-	-	-	-	-	-	-	
$J_{6\ 12}$	-	-	-	-	-	-	-	-	-	7.3
$J_{7\ 8}$	5 1	5.8	10 0	10.1	ca 10	10 1	11.4	12.0	11.8	11.2
$J_{8\ 13}$	9.5	10.1		9.5	10.0	10.1	10.2	10.1	9.9	
$J_{13\ 14}$	15.3	16.7	12.8	15.6	15.4	15.9	15.3	15.0	15 1	16.6
$J_{14\ 15a}$	5 0	5.0	4.9	4 9	4.9	4.9	4.4	4.2	4.1	3 9
$J_{14\ 15b}$	10.3	12.2	10.9	10.8	10.4	10.6	11.1	11.0	11.0	11 2
$J_{16\ 16\ CH3}$	6 3	6.7	6.7	6.7	6.3	4.9	7.0	4.9	6 9	
$J_{16\ 17a}$	1.7	2 6	3.1	3.2	2.4	2.6	2 3		2.5	
$J_{16\ 17b}$	1 8	2.6	2 6	2 9	3.1	2.5			2.5	
$J_{17a\ b}$	14 6	13.4	14.4	13.4	14.2	13.2	13.3		13 2	
$J_{19\ 20}$	16.7	16.7	16.8	16.5	16.7	18.2	16.8	16.8	16.5	
$J_{19\ 21}$	ca.0	ca.0	2.1	1.9	1.8	ca.0	2.4	2.3	2.2	
$J_{20\ 21}$	ca.0	ca.0	2.1	2.1	2.0	ca.0	2 1	2.2	1.9	
$J_{7\ 7\text{-}OH}$	-	-	6.0	6.1	6.8	6.7	4.9	4.7		5.1
$J_{21\ 21\ OH}$	-	6.1	-	6.3	-	6.1	-	6.6		

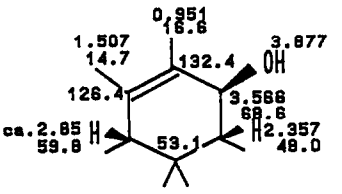
Table II ^{13}C NMR Data of Cytochalasins

100 MHz ^{13}C-NMR chemical shifts (ppm) in DMSO-d_6

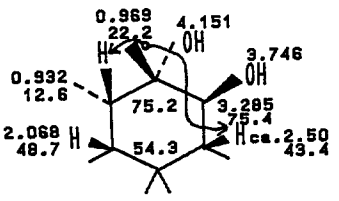
carbon	epoxy-cytochalasin H (1)	epoxy-cytochalasin J (2)	cytochalasin H (3)	cytochalasin J (4)	cytochalasin N (5)	cytochalasin O (6)	cytochalasin P (7)	cytochalasin Q (8)	cytochalasin R (9)	cytochalasin S (10)
1	174.3 (s)	176.2 (s)	174.1 (s)	176.5 (s)	174.5 (s)	176.6 (s)	174.5 (s)	175.5 (s)	176.0 (s)	174.8 (s)
3	53.3 (d)	53.3 (d)	52.9 (d)	53.2 (d)	49.1 (d)	48.5 (d)	52.6 (d)	52.4 (d)	52.4 (d)	52.7 (d)
4	48.6 (d)	48.9 (d)	47.9 (d)	48.1 (d)	60.0 (d)	59.8 (d)	48.8 (d)	48.7 (d)	47.3 (d)	55.5 (d)
5	35.9 (d)	36.2 (d)	31.8 (d)	33.6 (d)	125.5 (s)	126.4 (s)	37.8 (d)	37.9 (d)	38.2 (d)	72.1 (s)[a]
6	58.7 (s)	57.0 (s)	151.0 (s)	151.9 (s)	133.2 (s)	132.4 (s)	75.1 (s)	75.2 (s)	71.5 (s)	49.0 (d)
7	62.4 (d)	63.0 (d)	70.6 (d)	71.0 (d)	68.3 (d)	68.6 (d)	75.5 (d)	75.4 (d)	72.5 (d)	72.2 (d)
8	44.7 (d)	43.5 (d)	46.3 (d)	45.3 (d)	48.5 (d)	48.0 (d)	45.4 (d)	43.4 (d)	41.5 (d)	45.1 (d)
9	53.5 (s)	55.2 (s)	51.9 (s)	53.8 (s)	51.4 (s)	53.1 (s)	52.9 (s)	54.3 (s)	54.2 (s)	54.7 (s)
10	44.9 (t)	45.0 (t)	43.1 (t)	44.1 (t)	43.9 (t)	44.7 (t)	43.4 (t)	43.0 (t)	44.2 (t)	42.0 (t)
11	12.0 (q)	12.2 (q)	13.0 (q)	13.7 (q)	14.4 (q)	14.3 (q)	12.8 (q)	12.6 (q)	12.7 (q)	25.3 (q)
12	19.3 (q)	19.5 (q)	111.4 (t)	111.7 (t)	18.5 (q)	18.6 (q)	22.4 (q)	22.2 (q)	24.5 (q)	16.9 (q)
13	128.5 (d)	130.1 (d)	128.8 (d)	131.2 (d)	129.0 (d)	130.8 (d)	128.2 (d)	127.6 (d)	127.9 (d)	127.8 (d)
14	134.1 (d)	133.5 (d)	134.6 (d)	134.8 (d)	134.2 (d)	133.9 (d)	135.8 (d)	135.5 (d)	134.2 (d)	135.5 (d)
15	42.6 (t)	42.8 (t)	44.0 (t)	43.6 (t)	43.2 (t)	43.4 (t)	42.9 (t)	42.9 (t)	43.0 (t)	43.0 (t)
16	27.6 (d)	27.8 (d)	27.8 (d)	28.3 (d)	27.7 (d)	27.9 (d)	28.2 (d)	28.1 (d)	27.9 (d)	28.0 (d)
17	53.7 (t)	53.8 (t)	53.9 (t)	54.2 (t)	54.1 (t)	54.0 (t)	53.3 (t)	53.2 (t)	53.4 (t)	53.5 (t)
18	72.2 (s)	72.6 (s)	72.4 (s)	73.2 (s)	72.4 (s)	72.8 (s)	72.3 (s)	72.4 (s)	72.4 (s)	72.3 (s)[b]
19	124.5 (d)	129.1 (d)	125.4 (d)	129.3 (d)	125.0 (d)	129.4 (d)	136.8 (d)	135.5 (d)	136.0 (d)	136.5 (d)
20	138.7 (d)	137.6 (d)	138.1 (d)	137.2 (d)	138.7 (d)	137.6 (d)	126.4 (d)	131.8 (d)	131.3 (d)	129.8 (d)
21	75.4 (d)	73.7 (d)	76.7 (d)	75.2 (d)	75.1 (d)	73.4 (d)	77.6 (d)	75.5 (d)	75.2 (d)	74.2 (d)
22	26.1 (q)	26.2 (q)	26.2 (q)	26.7 (q)	26.2 (q)	26.4 (q)	26.3 (q)	26.2 (q)	26.2 (q)	26.2 (q)
23	30.5 (q)	31.1 (q)	30.9 (q)	31.6 (q)	30.6 (q)	31.2 (q)	32.1 (q)	32.4 (q)	31.9 (q)	31.9 (q)
1'	137.1 (s)	137.5 (s)	137.3 (s)	137.9 (s)	137.8 (s)	138.2 (s)	137.2 (s)	137.4 (s)	137.1 (s)	136.7 (s)
2'6'	129.6 (d)	129.9 (d)	129.7 (d)	130.5 (d)	129.4 (d)	129.8 (d)	130.0 (d)	130.1 (d)	129.9 (d)	130.2 (d)
3'5'	128.4 (d)	128.3 (d)	128.4 (d)	128.7 (d)	128.6 (d)	128.5 (d)	127.5 (d)	127.8 (d)	127.9 (d)	127.9 (d)
4'	126.5 (d)	126.5 (d)	126.5 (d)	127.0 (d)	126.7 (d)	126.6 (d)	128.4 (d)	126.0 (d)	126.2 (d)	126.3 (d)
21-Ac	170.0 (s)		170.1 (s)		170.4 (s)		169.2 (s)			
	20.5 (q)		20.6 (q)		20.4 (q)		20.5 (q)			

a,b) Assignments may be interchanged.

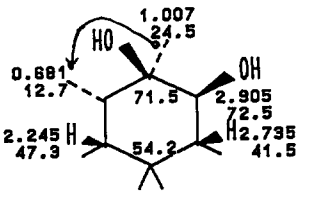




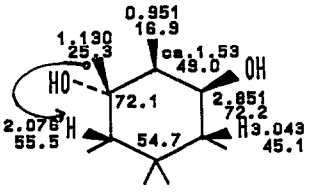
(6) cytochalasin O



(8) cytochalasin Q



(9) cytochalasin R



(10) cytochalasin S

CHART 2 The Conformation and the ^{1}H and ^{13}C NMR data of the Cyclohexane Moiety of Cytochalasins O, Q, R, and S

as described in the experimental section) and the positive first Cotton effect of the 6,7-dibenzoate (Fig. 1) showed the positive exciton chirality of the groups, indicating the α configuration of the C_6-hydroxyl. Thus, P (7) and Q (8) were proved to be 6α,7β-glycol type compounds, the first such compounds among the more than forty cytochalasans so far characterized.[3]

From the molecular formulae and the spectral data, cytochalasins R (9) and S (10) were suspected to be isomers in the cyclohexane moiety of cytochalasin Q (8). In the case of R (9), ^{1}H and ^{13}C NMR data including those obtained by COSY, C-H COSY, and C-H long range COSY revealed the bond connection from the C_{10}-protons to C_5-methyl and from the C_7-hydroxyl to the macrocyclic ring beyond C_8 and, accordingly, the presence of quaternary carbon at C_6 bearing hydroxyl and methyl groups. The NMR data also suggested that the cyclohexane moiety adopted the boat conformation (Chart 2) as in other cytochalasans.[2] The α configuration of the C_5-methyl group and the β configuration of the C_7-hydroxyl group were shown by the coupling constants (J_{4-5} 4.9 hz and J_{7-8} 11.7 Hz). The NOE observed between the C_5-methyl and C_6-methyl groups suggested α configuration of the C_6-methyl group. Thus, the structure of cytochalasin R (9) was proved to be the epimer of Q (8) at the C_6-position.

Similar examination of cytochalasin S (10) revealed the connection from the C_{10}-protons to the C_4-proton and from the sec-C_6-methyl group to the macrocyclic ring beyond C_8, and the presence of a quaternary carbon atom at C_5 bearing hydroxyl and methyl groups. In order to avoid overlappings of the signals, the spectra taken both in $CDCl_3$ and in DMSO were compared Since the NMR data

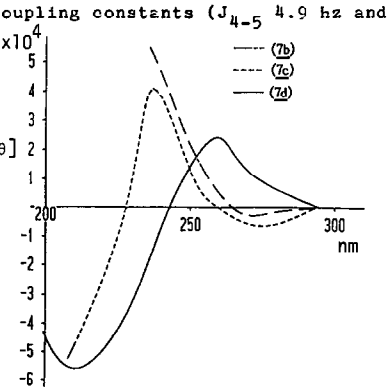


Fig. 1 CD Spectra of Cytochalasin P 7-Mono- (7b), 7,18-Di- (7c), and 6,7,18-Tri-benzoate (7d)

suggested that the cyclohexane moiety existed in the same conformation as in the other compounds (Chart 2), the coupling constants (J_{7-8} 12 Hz and J_{6-7} 8.2 Hz) showed β configuration of the C_7-hydroxyl group and the cis-configuration of the C_6-methyl and C_7-hydroxyl groups, respectively. On irradiation of the C_6-proton, couplings of the proton with the C_6-methyl protons and the C_7-proton were confirmed, while NOEs were observed at the C_{21}- and C_4-protons on irradiation of the C_5-methyl protons. Thus, the C_5-methyl was found to be β and the 5α,7β-diol stereochemistry of the cyclohexane moiety of the molecule (**10**) was suggested.

More than forty cytochalasans so far characterized have a 6,7-ene, 6,7-epoxide, 5(6)-en-7β-ol, or 6(12)-en-7β-ol structure in the cyclohexane (cyclohexene) moiety of the molecules.[3] Cytochalasins P (**7**), Q (**8**), R (**9**), and S (**10**) having 6α,7β-diol, 6β,7β-diol, and 5α,7β-diol structures are the first such compounds to be isolated among the cytochalasans. It is noteworthy that these novel compounds exhibited weaker cytotoxicity to mammalian cells and inhibition of capping.[17] The results of biological testing and further studies on the structure-activity relationship will be reported in a forthcoming paper.

Experimental

All melting points were determined on a Yanagimoto MP micromelting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a JEOL GX-400 (^1H 400 MHz and ^{13}C 100 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (δ) and coupling constants (J) in Hz. MS were taken on JEOL JMS-D300 and JEOL JMS-HX100 instruments. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a JASCO A-102 infrared spectrophotometer. The $[\alpha]_D$ values were measured with a JASCO DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20 spectropolarimeter. Kieselgel 60 F_{254} (Merck) precoated plates were used for thin-layer chromatography (TLC). Column chromatography was carried out on 70-230 mesh silica gel (Merck). HPLC was carried out by using a Waters M45J pump with an Oyo-Bunko Uvilog 7 UV detector and a Shodex RI SE-12 detector.

Isolation of the Metabolites of *Phomopsis* sp. (68-GO-104)

The mold was incubated in stationary culture on sterilized wheat (150 g x 200) at 26° C for 20 days. The moldy wheat was extracted three times with CH_2Cl_2 for 24 hrs at room temperature. The extract (232 g) was chromatographed over silica gel using a gradient system of hexane-acetone as the developing solvents to afford fractions 1 (hexane-acetone, 5 1), 2 (3.1), 3 (2.1), 4 (1:1) and 5 (1 2). The extract (8.9 g) obtained by the concentration of Fr. 1 was purified by silica gel column chromatography using $CHCl_3$-ethyl acetate (4.1) as the developer and by HPLC on Nucleosil 50-5 and oxalic acid-treated Nucleosil 50-5 columns using $CHCl_3$-ethyl acetate (2:1) and $CHCl_3$ as the developers, respectively, to give (3S,4S)-cis-(+)-4-hydroxymellein[8] (36 mg), colorless needles, mp 124-125°C ($CHCl_3$) (lit.[8] mp 118-119°C), $[\alpha]_D^{23}$ +31.4°; MS: 194.0589 (M^+, calcd for $C_{10}H_{10}O_4$, 194.0579), 150, 122, 121, 65, 43, UV λ_{max}^{MeOH} nm (ε). 311, 244, 210 (3140, 4000, 8540), IR ν_{max}^{KBr} cm^{-1}: 3400, 3200, 1660, 1620, 1462, 1240, ^1H NMR ($CDCl_3$) δ 1.55 (3H, d, J=6.6 Hz, 3-CH_3), 2 12 (1H, s, 4-OH), 4 57 (1H, d, J=1.8 Hz, 4-H), 4.70 (1H, dq, J=1.8, 6.6 Hz, 3-H), 6.93 (1H, 5-

H), 7.02 (1H, 7-H), 7.53 (1H, 6-H), 10.9 (1H, 8-OH); ^{13}C NMR ($CDCl_3$) δ. 16.0 (q, 3-CH_3), 67.2 (d, 4-C), 78.2 (d, 3-C), 106.8 (s, 9-C), 118.2 (d, 5-C), 118.5 (d, 7-C), 136.7 (d, 6-C), 140.5 (s, 10-C), 162.1 (s, 8-C), 169.1 (s, 1-C); CD (MeOH) $\Delta\varepsilon^{25}$ (nm): -0.55 (315), 0 (277), +3.62 (250); and trans-4-hydroxymellein[8] (43 mg), colorless needles, mp 130-131°C ($CHCl_3$), $[\alpha]_D^{23}$ +23.3°, MS. 194.0582 (M^+, calcd for $C_{10}H_{10}O_4$, 194.0579), 150, 122, 121, 65, 43; UV λ_{max}^{MeOH} nm (ε). 314, 245, 214 (4170, 5220, 1100); IR ν_{max}^{KBr} cm^{-1} 3400, 3200, 1660, 1620, 1462, 1240, ^{1}H NMR ($CDCl_3$) δ 1.52 (3H, d, J=5.9 Hz, 3-CH_3), 2.27 (1H, d, J=6.4 Hz, 4-OH), 4.58-4.66 (2H, m, 3-H, 4-H), 7.02 (1H, 7-H), 7.03 (1H, 5-H), 7.27 (1H, 6-H), 11.0 (1H, 8-OH); ^{13}C NMR ($CDCl_3$) δ. 17.9 (q, 3-CH_3), 69.2 (d, 4-C), 79.9 (d, 3-C), 106.7 (s, 9-C), 116.2 (d, 7-C), 117.9 (d, 5-C), 136.9 (d, 6-C), 141.1 (s, 10-C), 162.0 (s, 8-C), 168.4 (s, 1-C). CD (MeOH) $\Delta\varepsilon^{25}$ (nm). +0.41 (315), 0 (277), +1.23 (265), 0 (255), -5.17 (242). The identities of these compounds were established by direct IR and TLC comparison with authenic samples.

The evaporation of fraction 2 gave precipitates of a mixture of two cytochalasans (38.6 g), which was fractionated by HPLC on Nucleosil 50-5 using hexane-acetone (5 2) as the developer to give epoxycytochalain H (**1**) (28.4 g), colorless powder of mp 122-123°C (hexane-acetone) (lit.[12] mp 128-130°C), mp 153-156°C(MeOH), $[\alpha]_D^{23}$ +26.0°(MeOH), UV λ_{max}^{MeOH} nm (ε) 208 (16400); IR ν_{max}^{KBr} cm^{-1}: 3380, 1738, 1680, 1225, 1110, 960, 740, 700, MS m/z: 493 (M^+), 475, 433, 415, 402, 343, 324, 270, 240, 120, 91; Anal Calcd for $C_{30}H_{39}NO_5 \cdot CH_3OH$, C, 70.87; H, 8.28, N, 2.68. Found C, 70.83; H, 8.25, N, 2.66; and a new cytochalasan, cytochalasin N (**5**)(2.1 g).

Fraction 3 (19.0 g) was purified by low-pressure liquid chromatography on a column of silica gel twice using $CHCl_3$-MeOH(25 1) and hexane-acetone (3:2) as the developers and by HPLC on Nucleosil 50-5 employing hexane-acetone (3 2) to give three cytochalasans: cytochalasin H (**3**) (380 mg), colorless powder of mp 250-251°C (ether) (lit.[9] 258-263°C), $[\alpha]_D^{23}$ +32.5° (MeOH), UV λ_{max}^{MeOH} nm (ε) 208 (17500), IR ν_{max}^{KBr} cm^{-1} 3425, 2900, 1735, 1680, 1430, 1400, 1370, 1230, 1100, 960, 900, 700, MS m/z· 493 (M^+), 433, 415, 402, 384, 324, 120, 91, 43, epoxycytochalasin J (**2**) (6.6 g), colorless powder of mp 125-126°C ($CHCl_3$), $[\alpha]_D^{23}$ +69.8° (MeOH), UV λ_{max}^{MeOH} nm (ε). 209 (14900), IR ν_{max}^{KBr} cm^{-1}: 3425, 2950, 1680, 1120, 970, 750, 700, MS m/z: 451 (M^+), 433, 415, 360, 342, 324, 270, 120, 91; and a new cytochalasan, cytochalasin O (**6**)(3.8 g).

Fraction 4 (11.0 g) was chromatographed through a column of silica gel twice using $CHCl_3$-MeOH (25:1) and hexane-acetone (2 1) and then purified by HPLC on a Nucleosil 50-5 column and eluting with hexane-acetone (3:2) and hexane-$CHCl_3$-MeOH (2:22.1) to give cytochalasin J (**4**)(2.0 g), colorless powder of mp 158-160°C ($CHCl_3$) (lit.[9] mp 161-165°C), $[\alpha]_D^{23}$+32.8°(MeOH), UV λ_{max}^{MeOH}nm (ε) 209 (13900), IRν_{max}^{KBr} cm^{-1}: 3400, 2950, 2900, 1680, 1600, 1270, 1120, 960, 900, 700, MS m/z 451 (M^+), 433, 415, 360, 342, 324, 120, 91, and a new cytochalasan, cytochalasin P (**7**)(201 mg).

The identities of epoxycytochalasin h (**1**), expoxycytochalasin J (**2**), cytochalasin H (**3**), and cytochalasin J (**4**) were confirmed by the direct TLC and IR comparisons with the authentic samples.[9,12]

Fraction 5 (8.7 g) was chromatographed on columns of silica gel using $CHCl_3$-MeOH (15 1) and then hexane-acetone (5 3) as the developers and subjected to HPLC on a Nucleosil 50-5 column and

eluting with hexane-acetone (5:4) first and then hexane-acetone (5:3) to give three new cytochalasans, cytochalasins R (**9**) (110 mg), S (**10**) (52 mg) and Q (**8**) (153 mg).

Cytochalasin N (5)

Colorless powder of mp 253-254°C from acetone, $[\alpha]_D^{23}$+85.4° (MeOH), UV λ_{max}^{MeOH}nm (ε). 208 (19800); IR ν_{max}^{KBr} cm^{-1}: 3400, 2725, 1690, 1470, 1235, 1150, 960, 700; MS m/z: 493 (M^+), 475, 457, 415, 397, 324, 306, 250, 120, 91; Anal Calcd for $C_{30}H_{39}NO_5.(CH_3)_2CO$ C, 71.84; H, 8.22; N, 2.54. Found: C, 71.80, H, 8.10, N, 2.74; ^1H and ^{13}C NMR (Tables I and II).

Cytochalasin O (6)

Colorless needles of mp 187-188°C from hexane-acetone and colorless powder of mp 170-172°C from ether, $[\alpha]_D^{23}$ +59.7°(MeOH), UV λ_{max}^{MeOH} nm (ε). 207 (20800), IR ν_{max}^{KBr} cm^{-1}: 3425, 1670, 1250, 1140, 960, 700, MS m/z: 451 (M^+), 433, 415, 342, 324, 306, 120, 91; Anal Calcd for $C_{28}H_{37}NO_4$ H_2O: C, 71.61; H, 8.37; N, 2.98. Found: C, 71.99; H, 8.11; N, 2.99; ^1H and ^{13}C NMR (Tables I and II).

Cytochalasin P (7)

Colorless powder of mp 117-118°C ($CHCl_3$), $[\alpha]_D^{23}$ -116.3° (MeOH), UV λ_{max}^{MeOH} (ε) 208 (15200), IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1730, 1680, 1370, 1230, 960, 700; MS m/z: 511.2944 (M^+, Calcd for $C_{30}H_{41}NO_6$, 511.2934), 493, 452, 434, 420, 402, 324, 120, 91; ^1H and ^{13}C NMR (Tables I and II).

Cytochalasin Q (8)

Colorless powder of mp 158-159°C (hexane-acetone), $[\alpha]_D^{23}$ -47.8° (MeOH), UV λ_{max}^{MeOH} nm (ε): 209 (48750); IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 1680, 1455, 1370, 962; MS m/z: 469.2808 (M^+, Calcd for $C_{28}H_{39}NO_5$, 469.2826), 451, 433, 417, 360, 324, 120, 91, ^1H and ^{13}C NMR (Tables I and II).

Cytochalasin R (9)

Colorless powder of mp 106-107°C (hexane-acetone), $[\alpha]_D^{23}$ -46.0° (MeOH), UV λ_{max}^{MeOH} nm (ε): 206 (23200), IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 1680, 1455, 1370, 962; MS m/z. 469.2810 (M^+, Calcd for $C_{28}H_{39}NO_5$, 469.2826), 451, 433, 415, 360, 324, 120, 91, ^1H and ^{13}C NMR (Tables I and II).

Cytochalasin S (10)

Colorless powder of mp 149-151°C (hexane-acetone), $[\alpha]_D^{23}$ -62.9° (MeOH), UV λ_{max}^{MeOH} (ε): 208 (13370); IR ν_{max}^{KBr} cm^{-1} 3350, 2900, 1680, 1445, 1362, 960; MS m/z 469.2792 (M^+, Calcd for $C_{28}H_{39}NO_5$, 469.2826), 451, 433, 415, 378, 360, 342, 306, 288, 270, 242, 216, 120, 91; ^1H and ^{13}C NMR (Tables I and II).

Acetylation of Epoxycytochalasin J (2) and Cytochalasin P (7)

Epoxycytochalasin J (**2**) (123 mg) was treated with pyridine (3.0 ml) and acetic anhydride (3.0 ml) for 3 hrs at room temperature and the precipitate formed by the addition of the reaction mixture to water was purified by HPLC to give epoxycytochalasin H (**1**) (51 mg). Its identity was established by TLC and ^1H NMR.

By the same procedure cytochalasin P 7-O-acetate (**7a**) (28 mg) was obtained from cytochalasin P (**7**) (30 mg), colorless powder of mp 128-130°C(hexane-acetone), $[\alpha]_D^{23}$-12.9°(MeOH), UV λ_{max}^{MeOH}nm (ε). 208 (17170), IR ν_{max}^{KBr} cm^{-1} 3450, 2920, 1720, 1685, 1370, 1230, 962, ^1H NMR (DMSO-d_6) δ 1.02 (d, J = 6.2, 5-CH_3), 1.10 (s, 6-CH_3), 1.11 (d, J = 7.0, 16-CH_3), 1.31 (s, 18-CH_3), 1.53 (m, 17a-H), 1.76 (m, 5-H, 15a-H), 1.84 (m, 16-H), 1.86 (m, 17b-H), 1.98 (m, 15b-H), 1.99 (m, 4-H), 2.01 (s, 21-

OAc), 2.22 (7-OAc), 2.47 (dd, J = 13.6, 9.9, 10a-H), 2.89 (dd, J = 12.5, 9.7, 8-H), 3.01 (dd, J = 13.6, 3.3, 10b-H), 4.30 (m, 3-H), 4.50 (d, J = 12.5, 7-H), 5.17 (dm, J = 15.1, 14-H), 5.36 (dd, J = 15.1, 9.7, 13-H), 5.46 (d, J = 16.5, 19-H), 5.48 (21-H, 18-OH), 5.79 (s, 6-OH), 5.98 (dd, J = 16.5, 3.0, 20-H), 7.18, 7.32, 7.25 (arom.-H), 7.30 (s, 2-H); ^{13}C NMR (DMSO-d_6) δ 12.5 (11-C), 23.1 (12-C), 20.6 (21-OCO<u>C</u>H$_3$), 20.7 (7-OCO<u>C</u>H$_3$), 26.4 (16-CH$_3$), 28.6 (16-C), 31.9 (18-CH$_3$), 38.3 (5-C), 42.6 (15-C), 43.8 (8-C), 46.3 (10-C), 51.3 (4-C), 52.9 (9-C), 53.4 (17-C), 53.6 (3-C), 74.3 (18-C), 74.8 (6-C), 78.1 (21-C), 80.7 (7-C), 124.8 (13-C), 126.8 (20-C), 136.7 (19-C), 137.7 (14-C), 169.8 (21-O<u>C</u>OCH$_3$), 172.7 (7-O<u>C</u>OCH$_3$), 173.7 (1-C); MS <u>m</u>/<u>z</u>: 553.2994 (M$^+$, Calcd for $C_{32}H_{43}NO_7$, 553.3027), 535, 494, 416, 398. 120, 91, 43.

Deacetylation of Epoxycytochalasin H (1) and Cytochalasins H (3), N (5) and P (7)

i) Cytochalasin H (3) (33 mg) was dissolved in 5% KOH-MeOH (5 ml) and kept at 20°C for 4 hr under stirring. The reaction mixture was put into water, neutralized with HCl and extracted with $CHCl_3$. The extract was purified by HPLC to give cytochalasin J (4) (14 mg). In the same way, cytochalasin O (6) (61 mg) was obtained from N (5) (92 mg).

ii) Epoxycytochalasin H (1) (42 mg) in <u>tert</u>-BuOH (5 ml) was treated with 0.5 N NaOH solution (3 ml). The reaction mixture was stirred for 1 hr at room temperature, neutralized with HCl, and extracted with $CHCl_3$. The extract was purified by HPLC to give epoxyytochalasin J (2) (34 mg).

iii) Cytochalasin P (7) (54 mg) in acetonitrile (4 ml) was treated with 5% NaOH solution (4 ml) and the reaction mixture was stirred at 40° C for 2 hrs. After acidification, the reaction mixture was extracted with $CHCl_3$ and purified by HPLC to give Q (8) (40 mg).

Cleavage of the Epoxide Ring of Epoxycytochalasins H (1) and J (2)

i) Epoxycytochalasin H (1) (245 mg) was dissolved in 90% HOAc (14 ml) and the solution was kept at 45°C for 40 mins. The reaction mixture was poured into water and the precipitate formed was collected and washed with acetone and MeOH to give cytochalasin N (5) (101 mg), which was identified by ^{1}H NMR and TLC. The mother liquor was neutralized with ammonia, extracted with $CHCl_3$ and purified by HPLC to give cytochalasin H (3) (18 mg), which was identified as above. By the same procedure cytochalasin O (6) (18 mg) and J (4) (6 mg) were obtained from epoxycytochalasin J (2) (67 mg).

ii) Epoxycytochalasin H (1) (53 mg) and aluminum isopropoxide (41 mg) were refluxed in anhydrous toluene (10 ml) at 150° C for 8 hrs.[18] The reaction mixture was neutralized with HCl and extracted with ether The extract was purified by HPLC to give cytochalasin H (3) (30 mg).

iii) To a solution of epoxycytochalasin H (1) (51 mg) in DMSO (6 ml), 1N H_2SO_4 (3 ml) was added dropwise and the resultant solution was stirred for 30 mins at room temperature. The reaction mixture was neutralized by adding 10% $NaHCO_3$ and extracted with ether and the extract was purified by HPLC to give cytochalasin N (5) (43 mg).

iv) To a solution of epoxycytochalasin H (1) (45 mg) in acetonitrile (3 ml), 0.05 M CF_3CO_2H (2 ml) was added and the reaction mixture was kept at 70°C for 1 min,[15] neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was purified by HPLC to give cytochalasins P (7) (15 mg), N (5) (14 mg) and H (3) (5 mg)

Benzoylation of Cytochalasin P (7)

i) Benzoyl chloride (2 ml) was added to a solution of cytochalasin P (**7**) (25 mg) in pyridine (2 ml). The reaction mixture was stirred for 1 hr at room temperature, charged on Chemcosep Si-B and eluted with hexane-acetone. The eluate with hexane-acetone (2 1) was purified by HPLC using Nucleosil 50-5 as the absorbant and hexane-acetone (3:2) as the developer to give cytochalasin P 7-O-monobenzoate (**7b**) (21 mg), colorless powder of mp 136-137°C, UV λ_{max}^{EtOH} nm (ε). 229 (8330); IR ν_{max}^{KBr} cm^{-1}. 3440, 2910, 1690, 1450, 1370, 1280, 1230, 1115, 962; CD (Fig. 1), ^{1}H NMR (DMSO-d_6) δ 1.04 (d, J = 6.5, 16-CH_3), 1.20 (d, J = 7.1, 5-CH_3), 1.14 (s, 6-CH_3), 1.33 (s, 18-CH_3), 1.53 (m, 17a-H), 1.77 (m, 15a-H), 1.84 (m, 16-H), 1.89 (m, 17b-H), 1.93 (m, 5-H), 2.03 (m, 15b-H), 2.05 (m, 4-H), 2.24 (s, 21-OAc), 2.47 (dd, J = 13.6, 10.0, 10a-H), 3.06 (dd, J = 13.6, 3.7, 10b-H), 3.12 (od, J = 12.7, 9.7, 8-H), 3.97 (s, 18-OH), 4.43 (odd, J = 10.0, 5.5, 3.7, 3-H), 4.64 (d, J = 12.7, 7-H), 5.32 (ddd, J = 15.1, 10.7, 4.3, 14-H), 5.48 (od, J = 15.1, 9.7, 13-H), 5.51 (m, 19-H), 5.53 (m, 21-H), 5.73 (s, 6-OH), 6.02 (m, 20-H), 7.19-7.97 (m, 2-H, arom.-H); ^{13}C-NMR (DMSO-d_6) δ 12.8 (11-C), 23.1 (12-C), 20.8 (21-OCO<u>C</u>H_3), 26.5 (16-CH_3), 28.8 (16-C), 32.1 (18-CH_3), 38.7 (5-C), 42.7 (15-C), 44.2 (8-C), 46.5 (10-C), 51.7 (4-C), 53.1 (9-C), 53.4 (17-C), 53.7 (3-C), 74.4 (18-C), 75.1 (6-C), 78.3 (21-C), 82.2 (7-C), 125.4 (13-C), 126.9 (20-C), 136.8 (19-C), 137.9 (14-C), 168.5 (7-O<u>C</u>OC_6H_5), 169.8 (21-O<u>C</u>OCH$_3$), 173.6 (1-C), MS m/z. 615.3249 (M^+, Calcd for $C_{37}H_{45}NO_7$, 615.3196), 597, 556, 415, 397, 342, 324.

ii) Benzoyl chloride (2 ml) was added to a solution of cytochalasin P (**7**) (69 mg) in pyridine (2 ml) and benzene (2 ml). The mixture was refluxed for 1 hr, and, after cooling, charged on Chemcosep Si-B. The eluate with hexane-acetone (5.1) was purified by HPLC on a Nucleosil 50-5 column using hexane-acetone (3.1) to give cytochalasin P 7,18-O-dibenzoate (**7c**) (31 mg), colorless powder of mp 139-141°C(hexane-acetone), UV λ_{max}^{EtOH} nm (ε) 272, 227 (1660, 23200), IR ν_{max}^{KBr} cm^{-1}: 3440, 2900, 1685, 1445, 1365, 1275, 1220, 1105, 952; CD (Fig 1), ^{1}H NMR (DMSO-d_6) δ 1.13 (d, J=6 7, 16-CH_3), 1 14 (s, 6-CH_3), 1.19 (d, J=7.1, 5-CH_3), 1.71 (s, 18-CH_3), 1.79 (m, 15a-H), 1.85 (m, 16-H), 1.89 (m, 5-H), 1.92 (m, 17a-H), 2.04 (m, 15b-H), 2.14 (m, 4-H), 2.16 (m, 17b-H), 2.22 (s, 21-COCH_3), 2.53 (od, J=13.5, 9.9, 10a-H), 3.07 (dd, J=3.5, 3.5, 10b-H), 3.13 (od, J=12 6, 10 0, 8-H), 4.45 (ddd, J=9 9, 5.5, 3.5, 3-H), 4 60 (d, J=12.6, 7-H), 5.32 (ddd, J=15.5, 10.7, 4.4, 14-H), 5.54 (dd, J=15.5, 10.0, 13-H), 5.61 (dd, J=3.0, 2 3, 21-H), 5.75 (dd, J=16.6, 2.3, 19-H), 6.06 (dd, J=16 6, 3.0, 20-H), 6.12 (s, 6-OH), 7.21-8.04 (m, 2-NH, arom.-H), ^{13}C NMR (DMSO-d_6) δ. 12.7 (11-C), 20.8 (21-OCO<u>C</u>H_3), 23.0 (12-C), 25.8 (16-CH_3), 27.2 (18-CH_3), 28.8 (16-C), 38.6 (5-C), 42.5 (15-C), 44.1 (8-C), 46.3 (10-C), 51.4 (4-C), 51.6 (17-C), 53.3 (9-C), 53.7 (3-C), 75.0 (6-C), 78.0 (21-C), 82.0 (7-C), 85.1 (18-C), 125.8 (20-C), 126.0 (13-C), 135.2 (19-C), 137.1 (14-C), 165.3 (18-O<u>C</u>OC_6H_5), 168.5 (7-O<u>C</u>OC_6H_5), 169 6 (21-O<u>C</u>OCH$_3$), 173.9 (1-C). <u>Anal</u> Calcd for $C_{44}H_{49}NO_8 \cdot 1/2\ H_2O$ C, 72.54, H, 6.92, N, 1 92, Found C, 72 65, H, 7.01, N, 1.84, and cytochalasin P 6,7,18-<u>O</u>-tribenzoate (**7d**) (12 mg), colorless powder of mp 151-153°C (hexane-acetone), UV λ_{max}^{EtOH} (ε) 229 (24900), IR ν_{max}^{KBr} cm^{-1} 3450, 2940, 1722, 1690, 1455, 1375, 1275, 1225, 1175, 1115, 960, CD (Fig. 1), ^{1}H NMR (DMSO-d_6) δ 0 37 d, J=7.0, 5-CH_3), 0.99 (s, 6-CH_3), 1.05 (d, J=7.0, 16-CH_3), 1.23 (m, 17a-H), 1.61 (m, 15a-H), 1.72 (s, 18-CH_3), 1 74 (m, 16-H), 1.77 (m, 5-H), 1.78 (m,15b-H), 2.00

(m, 4-H), 2.04 (m, 17b-H), 2.32 (s, 21-COCH$_3$), 2.91 (dd, J=10.4, 9.9, 8-H), 2.99 (dd, J=12.8, 10.4, 10a-H), 3.32 (dd, J=12.8, 2.7, 10b-H), 4.89 (m, 3-H), 4.89 (d, J=10.4, 7-H), 5.20 (ddd, J=15.6, 10.7, 4.9, 14-H), 5.65 (dd, J=15.6, 9.9, 13-H), 5.67 (dd, J=16.2, 2.1, 19-H), 5.75 (dd, J=16.2, 2.1, 20-H), 6.01 (dd, J=2.1, 2.1, 21-H), 7.26-8.02 (m, 2-NH, arom.-H), ^{13}C NMR (DMSO-d$_6$) δ 10.8 (11-C), 20.9 (21-OCOCH$_3$), 23.2 (12-C), 25.6 (16-CH$_3$), 25.7 (18-CH$_3$), 28.2 (16-C), 36.0 (5-C), 41.3 (8-C), 42.3 (15-C), 43.5 (10-C), 44.3 (4-C), 52.3 (17-C), 54.6 (9-C), 57.5 (3-C), 74.3 (21-C), 75.3 (6-C), 80.4 (7-C), 84.5 (18-C), 123.6 (20-C), 126.8 (13-C), 135.4 (19-C), 137.3 (14-C), 165.4 (18-OCOC$_6$H$_5$), 166.5 (6-OCOC$_6$H$_5$), 169.7 (21-OCOCH$_3$), 170.9 (7-OCOC$_6$H$_5$), 174.8 (1-C), MS m/z 823 (M^+), 701, 610, 263, 224, 105, 91, 77.

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