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Structure-Activity Relationships among Zygosporin Derivatives

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Cytochalasin D (zygosporin A) showed strong cytotoxic effect with a unique cytopathogenic change.

Further, cytochalasin D inhibited the growth of several rat tumors in a narrow chemotherapeutic index range owing to its irritating toxicity. More than 30 derivatives of zygosporin were synthesized, and structure-biology relationship was discussed for cytotoxicity and skin irritating activity. Among the derivatives, cytochalasin C has similar cytotoxicity to cytochalasin D, accompanied by a decreased lethal toxicity. It is disappointing, however, that the chemotherapeutic index of cytochalasin C is no different from that of cytochalasin D.

We have previously reported the isolation[2] and elucidation of the absolute stereostructures[3-5] of cytochalasin D (zygosporin A) and zygosporins D, E, F, and G. The compounds are isolated from the culture filtrate of *Zygosporium masonii* and they show characteristic cytotoxicity *in vitro*.

Though each of these antibiotics show strong growth inhibitory activity against cultured cells, their cytotoxicities (LD_{50}) range from 0.15 μg/ml to >10 μg/ml. This prompted us to undertake the present study, in which we investigate the relationship of each functional group in cytochalasin D (I) to its cytotoxicity.

During antitumor tests with cytochalasin D, we had found the intraperitoneal injection of cytochalasin D in mice gave rise to ascites as a side effect. We supposed that this is due to an irritating action of the antibiotic. It is reported[6] that chemical irritants induce an increased vascular permeability when injected intracutaneously, as indicated by exudation of circulating dye. A comparison of the cytotoxicities of natural zygosporins with their skin irritating activities suggests that these two biological properties are not related (see Table I). A further purpose of our present work is, therefore, to find a compound showing maximum cytotoxicity with least irritation.

This paper describes the synthesis of some derivatives of cytochalasin D and studies on their biological character.

Material and Method

Materials——Details of the preparation and properties of compounds (I) to (XXXVIII) are given in the Experimental section. Most of the compounds are sparingly soluble in water and so were suspended in physiological saline solution with the aid of 0.5% carboxy methyl cellulose or 2% gum arabic for *in vivo* tests. For the cytotoxicity tests, compounds were dissolved in methanol at a concentration of 1 mg/ml and subjected to serial ten-fold dilutions with the culture medium.

1) *a*, *b*) Location: *Fukushima-ku, Osaka, 553, Japan.*
2) S. Hayakawa, T. Matsushima, T. Kimura, H. Minato, and K. Katagiri, *J. Antibiotics*, **21**, 523 (1968).
3) Y. Tsukuda, M. Matsumoto, H. Minato, and H. Koyama, *Chem. Commun.*, **1969**, 41.
4) H. Minato and M. Matsumoto, *J. Chem. Soc.* (*C*), **1970**, 38.
5) H. Minato and T. Katayama, *J. Chem. Soc.* (*C*), **1970**, 45.
6) R.H. Steele and D.L. Wilhelm, *Brit. J. Exp. Path.*, **47**, 612 (1966).

Animals——Male Wistar strain rats weighing 80—100 g, and male DS strain Swiss albino mice weighing 18—20 g were used (supplied by our animal center, Aburahi, Shiga Prefecture).

Cytotoxicity Test——HeLa cells used in this experiment were grown in Eagle's minimum essential medium containing 10% bovine serum. For subcultivation, cells were removed from the glass with 0.03% trypsin and 0.04% ethylene diamine tetra-acetic acid. The cells were then resuspended in fresh medium, diluted to a concentration of 10^5 cells/ml, and implanted in a series of triplicate culture tubes.

Drugs were added to the cell cultures on the second day of cultivation. After two days of incubation with a drug, the cell population was measured by electronic cell counter.

The effective dose for 50% growth inhibition (ED_{50}) was determined by plotting the logarithmic curve of drug concentration against growth rate (Number of treated cells/Number of control cells).

Table I. Biological Activities of Zygosporin Derivatives

	Cytotoxicity ED_{50} (γ/ml)	Skin irritation infiltrated dye (γ/site)	Acute toxicity LD_{100} (mg/kg)
Cytochalasin D (I) (=Zygosporin A)	0.15	174	10
Zygosporin D (II)	0.4	184	10
Zygosporin E (III)	0.4	—	—
Zygosporin F (IV)	>10	—	—
Zygosporin G (V)	0.68	25	>50

Table II. Biological Activities of Zygosporin Derivatives

	Cytotoxicity ED_{50} (γ/ml)	Skin irritation infiltrated dye (γ/site)	Acute toxicity LD_{100} (mg/kg)
Isocytochalasin D (VI)	>10	—	>50
Cytochalasin D-diacetate (VII)	5.3	—	—
Zygosporin E-acetate (VIII)	>10	—	—
Zygosporin E-acetate epimer (IX)	10	—	—
X	1.6	105	20
XI	1.3	136	20
11-Dihydro-cytochalasin D (XII)	0.88	313	
11-Dihydro-zygosporin D (XIII)	0.96	104	
XIV	>10	—	—
XV	>10	—	—
XVI	0.2	339	—
XVII	0.93	35	50
XVIII	>10	13	50
XIX	2.7	33	50
XX	0.6	66	50
XXI	0.6	15	
XXII	1.8	—	
XXIII	1.1	—	
XXIV	7.2	12 (± 11.4)	
XXV	>10		
XXVI	10		
Dodecahydro-cytochalasin D (XXVII)	3.4		
Hexahydro-cytochalasin D (XXVIII)	0.23	312.2(± 58.5)	
Tetrahydro-cytochalasin D (XXIX)	0.19	296.2(± 62.6)	
Dihydro-cytochalasin D (XXX)	0.17	388 (±154.5)	
Tetrahydro-cytochalasin C (XXXI)	0.18	254 (± 37.4)	
Dihydro-cytochalasin C (XXXII)	0.7		
Dihydro-cytochalasin C-isomer (XXXIII)	0.8		
Cytochalasin C (XXXIV)	0.12	25.8(± 15.4)	>100
XXXV	1.1		
XXXVI	5.0	—	
XXXVII	8.0	10 (± 9.6)	

Skin Irritation Test——The method was described in previous publications.[7,8]

Lethal Toxicity——Test compounds were administrated intraperitoneally in mice, and observation made for 10 days.

Antitumor Test——The method was described previously.[9]

Result

The results are summarized in Tables I and II.

Cytotoxicity

First, the reproducibility of the cytotoxicity test (ED_{50}) was examined. From eight repeated experiments, the ED_{50} of cytochalasin D was calculated as 0.15 ± 0.048 mcg/ml (Fig. 1). This result confirmed the reproducibility of the assay method and allows discussion of structure–cytotoxicity relationships to be made.

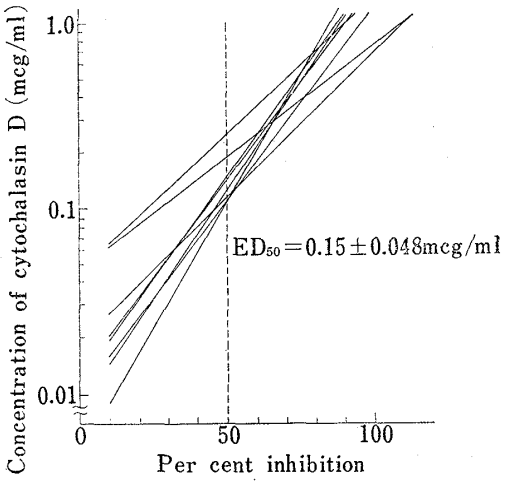


Fig. 1. Dose-response Curves for Cytochalasin D

(1) Hydroxy-Group at C-6——Comparison of the cytotoxicities of I and IV, clearly shows that the hydroxy-group at C-6 in I has a very favorable influence upon ED_{50}. Comparison of zygosporin E (III) with its C-6 acetates (VIII and IX), of XVII with XVIII, and of XVI with XI, shows that acylation of the hydroxy-group at C-6 weakens that cytotoxicity extremely. Comparison of X and XXXVI with I and XXX, respectively, shows that the activities of X and XXXVI are about one-tenth as strong as those of I and XXX, respectively. Therefore, it is concluded that presence of the hydroxy-group at C-6 is very important for the appearance of cytotoxicity.

(2) Carbonyl-Group at C-11——From comparison of I with XII and of II with XIII it is seen that reduction of the carbonyl at C-11 to a hydroxy-group has little effect on ED_{50}.

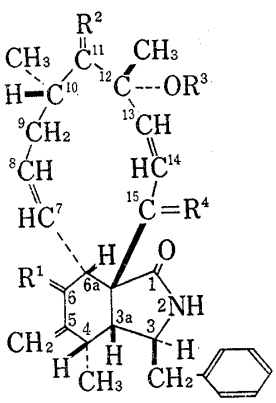
(3) Hydroxy-Group at C-12——The hydroxy-group at C-12 in IV, is replaced by an acetoxy-group in VII, a hydrogen–atom in VIII, and a chlorine-atom in XIV. Compound (XV) has no hydroxy-group at C-12 and has a C-12 (13) ethylenic double bond. As all of these compounds have an acetoxy-group at C-6, their cytotoxicities are very weak, and there is no advantage in comparing their ED_{50} values with one another. Compounds (I and III) having a hydroxy-group at C-6, showed similar ED_{50} values.

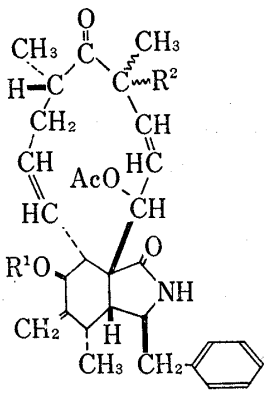
The substituent at C-12 is also assumed to have little effect on the cytotoxicity.

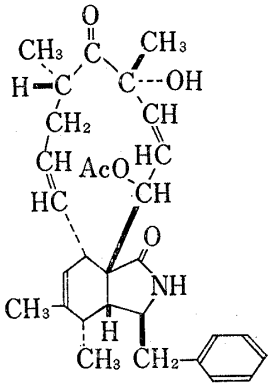
(4) Acetoxy-Group at C-15——Comparison of II with I, XIII with XII, and VI with IV, shows that there is no distinction in ED_{50} depending on whether there is an acetoxy-group or a hydroxy-group at C-15. However, compound (XI) having a carbonyl-group at C-15 showed stronger activity (ED_{50}; 1.3 μg/ml) than the C-15 hydroxy-derivative (VI) (ED_{50}; >10 μg/ml).

(5) 13-Substituted-15-oxo-derivatives——Since the 15-oxo-compound (XI) showed interesting activity, XI was hydrolyzed with an alkali in methanol to give the 6-hydroxy-15-oxo-derivative (XVI). The major product of this hydrolysis was not XVI however, but the 13-methoxy-ketone (XVII). As described later, this compound (XVII) showed only

7) M. Harada, M. Takeuchi, and K. Katagiri, *J. Antibiotics* (Tokyo), Ser, A, **20**, 369 (1967).
8) M. Harada, M. Takeuchi, T. Fukao, and K. Katagiri, *J. Pharm. Pharmacol.*, **23**, 218 (1971).
9) S. Matsuura, O. Shiratori, and K. Katagiri, *J. Antibiotics* (Tokyo), Ser. A, **17**, 234 (1964).



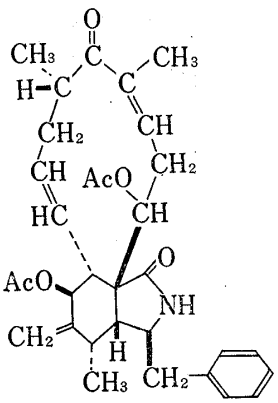




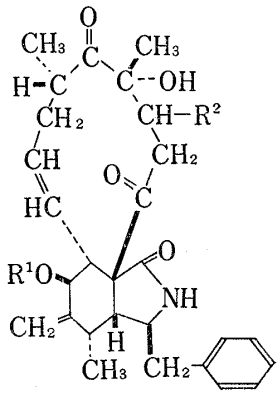
V

	R^1	R^2	R^3	R^4
I		=O	H	
II		=O	H	
IV		=O	H	
VI		=O	H	
VII		=O	Ac	
X	=O	=O	H	
XI		=O	H	=O
XII			H	
XIII			H	
XVI		=O	H	=O

	R^1	R^2
III	H	H
VIII	Ac	H
IX	Ac	H
XIV	Ac	Cl



XV



	R^1	R^2
XVII	H	$-OCH_3$
XVIII	Ac	$-OCH_3$
XIX	Ac	$-SC_2H_5$
XX	H	$-SC_2H_5$

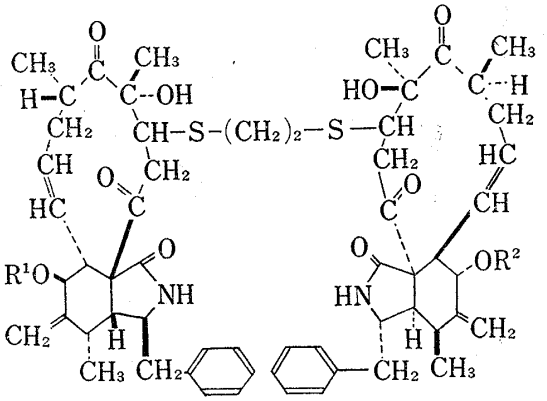
Chart 1

a weak skin irritation reaction. We therefore synthesized some derivatives having an S-alkyl substituent instead of an O-alkyl substituent at C-13, XIX, XX, XXI, XXII, and XXIII.

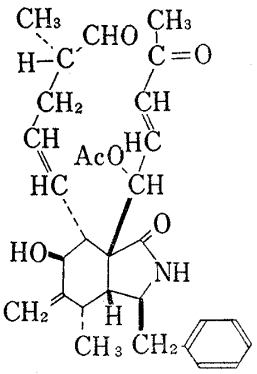
Comparison of the cytotoxicities of these compounds with those of the α,β-unsaturated ketones (XI and XVI) shows that the activities of the former are weaker than those of the latter though the differences are not great; *viz.* comparisons XI with XIX, XXI or XVIII, and XVI with XX or XVII. Moreover, among the S-alkyl derivatives, it is recognized that the hydroxy-group at C-6 is not the most important substituent for the appearance of the cytotoxicity; *viz.* comparisons XX with XXI, and XXII with XXI or XXIII.

(6) Benzene-ring——Comparison of dodecahydro-cytochalasin D (XXVII) with hexahydro-cytochalasin D (XXVIII) shows that the ED_{50} of XXVIII is about one-tenth that of XXVII. Thus the benzene ring is essential for high activity.

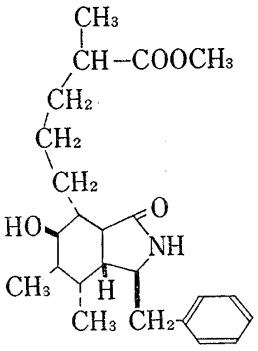
(7) Double Bonds in Eleven-membered Ring——The activity of dihydro-cytochalasin D (XXX) was compared with those of tehahydro-cytochalasin D (XXIX) and hexahydro-cytochalasin D (XXVIII); and the activity of cytochalasin C (XXXIV) (see later) was



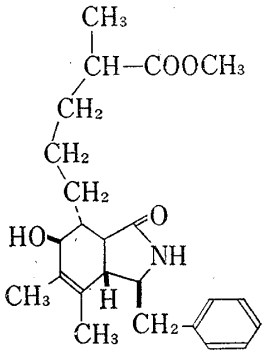
	R^1	R^2
XXI :	Ac	Ac
XXII :	H	H
XXIII :	H	Ac



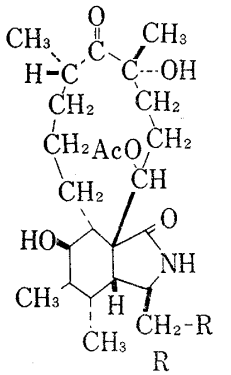
XXIV



XXV

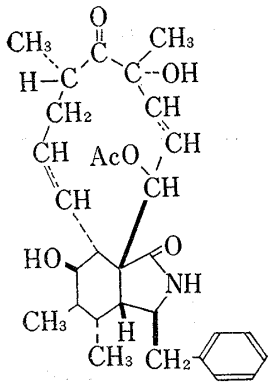


XXVI

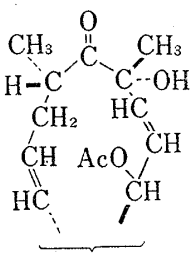


XXVII

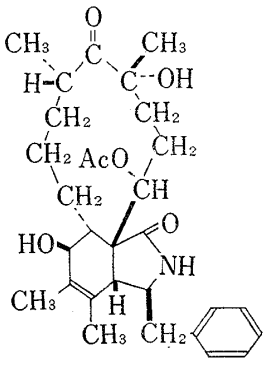
XXVIII



XXIX



XXX



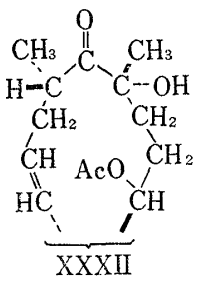
XXXI

Chart 2

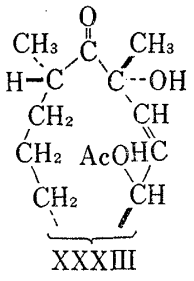
compared with these of dihydro-cytochalasins C (XXXII and XXXIII) and tetrahydro-cytochalasin C (XXXI). The results show that hydrogenation of the double bonds in the 11-membered ring has no effect on ED_{50}.

(8) **Double Bonds in the Six-membered Ring**——As shown by comparison of I with XXX, and of X with XXXV, hydrogenation of the exocyclic double bond at C-5 to a methyl-group has no effect on ED_{50}. Comparison of V with XXXVI reveals that a double bond at C-5 (6) has a favorable influence on ED_{50}.

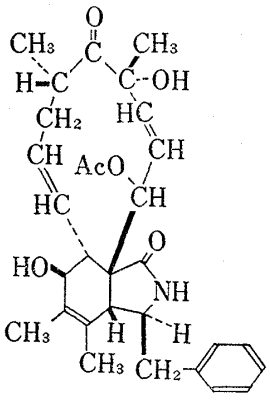
It is interesting to compare the effects of a double bond in different positions. Cytochalasin D (I) has an exocyclic double bond at C-5, and cytochalasin C (XXXIV) and V have



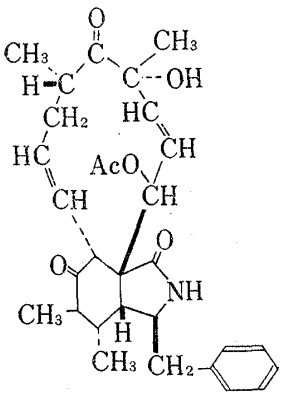
XXXII



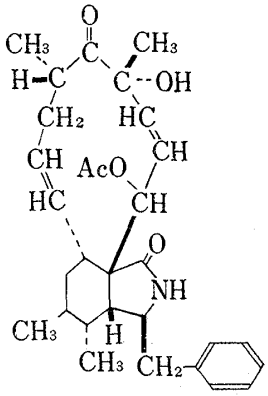
XXXIII



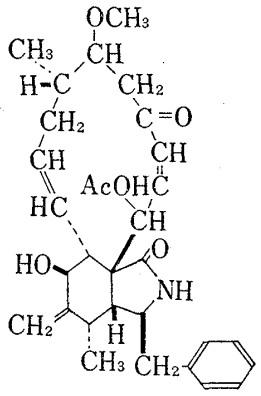
XXXIV



XXXV



XXXVI



XXXVII

Chart 3

an endocyclic double bond at C-4 (5) and C-5 (6) respectively. These compounds showed ED_{50} values of 0.15, 0.12, and 0.68 μg/ml, respectively. Therefore, there is no difference between the ED_{50} values of I and XXXIV, and it is assumed that the decreased activity in V is not caused by the position change of the double bond but by loss of the hydroxy-group at C-6.

(9) Skeleton of Cytochalasin——As it was clarified that hydrogenation of the double bonds at the six- or eleven-membered rings has no effect on ED_{50}, the effect of ring-opening of the 11-membered ring was investigated. The ED_{50} values of XXV, XXVI, and XXIV were all *ca.* 10 μg/ml, indicating that retention of the skeleton[10] of cytochalasin is an important requirement for cytotoxicity. It may be given as a conclusion that the most favorable derivatives are compounds having a hydroxy-group at C-6 and benzyl-group at C-3 in the cytochalasin skeleton.

Skin Irritating Toxicity

The effect of cytochalasin D on vascular permeability in rats was examined and the results are shown in Fig. 2. The minimal dosage to elicite the reaction was 0.2 γ/site. The response to the agent increased sharply as the dose increased. For the comparison of toxicity, 20 γ/site of compound was chosen. The increased vascular permeability was not inhibited by intraperitoneal injection of diphenhydramine (30 mg/kg) or colchicine (25 mg/kg).

10) The activity of XXXVII, having a 12-membered ring, was very weak.

As shown in the Tables I and II, cytochalasin D (I), zygosporin D (II), XVI, and XII, which have a hydroxy-group at C-6, all elicited a strong skin reaction. With the 6-oxo-derivative (X), however, the irritation value was decreased to one half, although the cytotoxicity was also weakened. Moreover, the products of hydrogenation of the double bonds in the 6- and 11-membered rings (XXX, XXIX, and XXVIII) showed stronger irritation than cytochalasin D (I). It is seen from these results that derivatives having a hydroxy-group at C-6 have an unfavourably strong skin irritation activity. As mentioned above, the 13-methoxy-15-oxo-derivative (XVII) was obtained by hydrolysis of XI. In spite of the fact that this compound has the C-6 hydroxy-group, its irritation value was only 35 μg, one-fifth—one-sixth that of I. As the 13-alkoxy-15-oxo-derivative (XVII) showed very weak skin irritation, we tested the stimulation activities of derivatives having an S-alkyl substituent at C-13. Compounds (XIX, XX, XXI, XXII, and XXIII) all showed weak skin irritation as shown in Table II. In particular, compound (XXI) has an irritation value of only 15 μg; though its cytotoxicity was about one-quarter as strong as that of I.

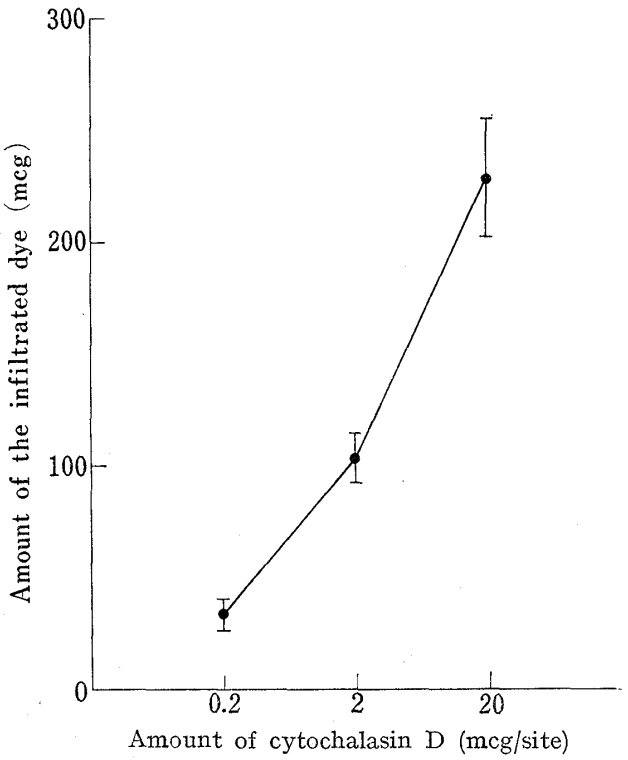


Fig. 2. Increased Vascular Parmeability caused by Cytochalasin D in the Rat

On the other hand, zygosporin G (V) showed a very weak irritation value of 25 μg. This compound has no exocyclic double bond at C-5 but has an endocyclic double bond at C-5 (6) in the six-membered ring. We assumed from this result that the endocyclic double bond in the six-membered ring may weaken the skin irritation. Accordingly, we wished to synthesize a compound having a hydroxy-group at C-6 and an endocyclic double bond at C-4 (5), that is, a derivative represented by the formula (XXXIV). Compound (XXXIV) has

Table III. Effect of Zygosporin Derivatives on Rat Ascites Hepatoma AH-130

Compound / Dose mg/kg/day	Cytochalasin D	XVII	XXII	XXIX	Cytochalasin C
40	—	—	—	—	0.19(卄)
20	—	—	—	—	0.83(−)
10	—	toxic	—	0.16(卄)	0.91(−)
5	toxic	0.81(−)	1.06(−)	0.76(−)	0.88(−)
2.5	0.42(+)	0.97(−)	0.96(−)	0.91(−)	—
1.25	0.56(±)	—	1.01(−)	—	—
0.63	0.71(±)	—	—	—	—
0.31	1.01(−)	—	—	—	—
Cytotoxicity, γ/ml	0.15	0.93	1.8	0.19	0.12

Treatment (*i.p.*) was started 24 hr after tumor inoculation and continued for 5 days. Antitumor activity was expressed as a tumor index, which was calculated from the ratio of total packed cell volume (TPCV) of the treated group to that of the control group.

already been isolated as cytochalasin C[11] from *Metarrhizium anisopliae* by the I.C.I. group, and its structure has been elucidated by them. As we could not isolate cytochalasin C (XXXIV) from *Zygosporium masonii*, we obtained it by chemically isomerizing cytochalasin D (I). On isomerization of I under hydrogenation conditions with 10% Pd-C, cytochalasin C (XXXIV) was obtained together with a small amount of another isomer (XXXV).

As expected, cytochalasin C (XXXIV) showed a strong cytotoxicity value of 0.12 μg/ml and a weak irritation value of 25.8 μg.

Antitumor Activity

Five compounds were selected for antitumor test on rat ascites hepatoma AH-130. The results are shown in Table III. Among the compounds, 3 showed growth inhibition of ascites tumor cells. It is disappointing that the antitumor active doses are very close to toxic doses; especially, cytochalasin C inhibits the ascites tumor only at toxic doses.

Discussion

In 1967 Carter[12] reported that cytochalasins have a cytotoxic effect on mammalian cells. Since then a large number of *in vitro* studies have been carried out on the biological activities of these antibiotics, though there have been very few studies *in vivo*. The antitumor activity of cytochalasin D is therefore very interesting. In the present experiments most compounds were tested by irritation test and cytotoxicity test, as these two tests need only small samples and are valuable for examining the biological activities. When a derivative showed cytotoxicity at under 10 γ/ml, unique cytopathological changes were observed without exception. Cytochalasins C and D are about 5 times as active as Phomin (cytochalasin B) in our assay system. Investigation of structure–cytotoxicity relationships showed that a hydroxy-group at C-6 and a benzyl group at C-3 in the skeleton are essential for activity. The criteria of one of our initial aims, to obtain a cytotoxic compound with decreased irritating effect, were met by cytochalasin C, synthesized from cytochalasin D.

Skin irritating toxicity and lethal toxicity were closely related among the tested compounds in these series of experiments. As already reported,[13] cytochalasin D is mainly active against rat tumors; so comparison of antitumor activity was performed with rat ascites hepatoma AH-130. Several compounds which showed stronger cytotoxicities were selected for the antitumor test. Three out of five compounds showed growth inhibition of tumor cells. This result suggests that the unique cytopathogenic effect is not necessarily correlated with the antitumor activity. The results with cytochalasin C were particularly disappointing; despite the fact that its lethal toxicity is ten times less that of cytochalasin D while strong cytotoxicity is retained, the chemotherapeutic index of cytochalasin C is no different from that of cytochalasin D. Further extensive studies are necessary to determine the antitumor mechanism of cytochalasins.

Experimental

Nuclear magnetic resonance (NMR) spectra were taken with a Varian A 60 spectrometer. Silica gel G (Merck) was used for thin-layer chromatography (TLC).

Cytochalasin D=Zygosporin A[4,5] (I), Zygosporin D[5] (II), Zygosporin E[5] (III), Zygosporin F[5] (IV), Zygosporin G[5] (V), Isocytochalasin D[4] (VI), Cytochalasin D-diacetate[4] (VII), Zygosporin E-acetate[5] (VIII), Zygosporin E-acetate Epimer[5] (IX), X[4], XI[4], 11-Dihydro-cytochalasin D[4] (XII). 11-Dihydro-zygosporin D (XIII)——A solution of cytochalasin D (I, 100 mg) in dry tetrahydrofuran (5 ml) was added dropwise to a suspension of $LiAlH_4$ (76 mg) in dry tetrahydrofuran (2 ml) with stirring in an ice-bath and stirred for

11) D.C. Aldridge, J.J. Armstrong, R.N. Speake, and W.B. Turner, *Chem. Commun.*, **1967**, 26; *idem*, *J. Chem. Soc.* (*C*), **1967**, 1667; D.C. Aldridge and W.B. Turner, *J. Chem. Soc.* (*C*), **1969**, 923.
12) S.B. Carter, *Nature*, **213**, 261 (1967).
13) K. Katagiri and S. Matsuura, *J. Antibiotics*, **24**, 722 (1971).

2 hr at room temperature. The mixture was decomposed by addition of water and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave a crystalline residue (98 mg) which was crystallized from EtOAc to give 11-dihydro-zygosporin D (XIII) as colorless prisms, mp 186—190°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3415, 1690, 1002, 982, 910. *Anal.* Calcd. for $C_{28}H_{37}O_5N$: C, 71.92; H, 7.98; N, 3.00. Found: C, 71.55; H, 7.50; N, 2.85.

XIV[5]**, XV**[5]**. XVI, XVII, and XVIII**——A solution of XI (200 mg) in 5% K_2CO_3–MeOH (10 ml) was left for 2.5 hr at room temperature and extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave a crystalline residue (190 mg). This residue was chromatographed on silica gel and purified by preparative TLC on silica gel to give XVI and XVII. Compound (XVI) was obtained as colorless prisms (18 mg), mp 127—131.5° (from EtOAC), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575, 3420, 1705—1690, 1620. *Anal.* Calcd. for $C_{28}H_{33}O_5N$: C, 72.54; H, 7.18; N, 3.02. Found: C, 72.54; H, 7.42; N, 2.75. Compound (XVII) was obtained as colorless prisms (135 mg), mp 191—196° (from acetone), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3440, 3420, 1700, 1094, 1005, 981, 912. *Anal.* Calcd. for $C_{29}H_{37}O_6N$: C, 70.28; H, 7.53; O, 19.37; N, 2.83. Found: C, 69.83; H, 7.48; O, 18.98; N, 2.95. Its acetate (XVIII) was obtained as a resinous product, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1730, 1700, 1097, 1005, 980, 910 by acetylation of XVII.

XIX and XX——Compound (XI, 300 mg) was added to a solution of KOH (333 mg) in EtSH (5.8 ml) and H_2O (1.5 ml) and left for 2 hr with stirring at room temperature. This mixture was extracted with ether and the extract was washed with 5% KOH and H_2O, dried (Na_2SO_4) and evaporated *in vacuo* to leave an amorphous powder (XIX, 333 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1732, 1705, 1118, 1014, 1002, 980, 914. Compound (XIX, 230 mg) was dissolved in 4.5% K_2CO_3–MeOH (8 ml) and left for 1 hr at room temperature. This was extracted with $CHCl_3$ and extract was washed with water, dried (Na_2SO_4), and evaporated to leave a residue (240 mg). This residue was chromatographed on silica gel to give XX, which was crystallized from acetone to give colorless prisms (161 mg), mp 182—186°, $[\alpha]_D^{23}$ −56.4° (±1.4°) (c=0.732 in dioxan), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3440, 3420, 1705, 1109, 1004, 982, 935, 914 (*Anal.* Calcd. for $C_{30}H_{39}O_5NS$: C, 68.54; H, 7.48; O, 15.22; N, 2.66; S, 6.10. Found: C, 68.61; H, 7.56; O, 14.88; N, 2.82; S, 6.32), and XVII (36 mg) and the starting material XIX (12 mg).

XXI, XXII, and XXIII——Compound (XI) (405 mg) was added to a mixture of ethane dithiol (7.1 ml) and 7% KOH (3 ml) and stirred vigorously for 17 hr at room temperature. The mixture was extracted with ether and the extract was washed with 5% KOH (four times) and water, dried (Na_2SO_4), and evaporated to leave a residue (335 mg). The residue was crystallized from acetone–*n*-hexane to give XXI (182 mg) as colorless prisms, mp 266—270° (decomp.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3210, 3090, 1732, 1700, 1113, 1017, 1004, 981, 914. *Anal.* Calcd. for $C_{63}H_{76}O_{12}N_2S_2$: C, 67.37; H, 6.93; O, 17.37; N, 2.53; S, 5.80. Found: C, 66.99; H, 6.90; O, 17.37; N, 2.45; S, 5.49.

Compound (XXI) (119 mg) was dissolved in 3.5% K_2CO_3–MeOH (12 ml) and left for 1.5 hr at room temperature. This was extracted with $CHCl_3$ and the extract was washed with water, dried (Na_2SO_4), and evaporated to leave a crystalline residue (124 mg). The residue was separated by preparative TLC into XXII (51.5 mg), which was crystallized from MeOH–benzene to give colorless needles, mp 250—256° (decomp.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3535, 3405, 3200, 3060, 1699, 1111, 1005, 982, 915 (*Anal.* Calcd. for $C_{58}H_{72}O_{10}N_2S_2$: C, 68.22; H, 7.12; N, 2.74. Found: C, 67.89; H, 7.20; N, 2.69), and XVII (49.5 mg) and a resinous product XXIII (4 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1700.

XXIV,[4] **XXV**,[4] **XXVI**,[4] **Dodecahydro-cytochalasin D**[4] **(XXVII), Hexahydro-cytochalasin D**[4] **(XXVIII), Tetrahydro-cytochalasin D**[4] **(XXIX). Dihydro-cytochalasin D (XXX)**——A solution of cytochalasin D (I) (100 mg) in EtOH (25 ml) was hydrogenated over 5% palladized barium carbonate (1 g) for 15 min at room temperature. The reaction product was applied on a thin-layer plate of silica gel and developed with a solvent system of benzene–MeOH (8: 1). The plate was developed five times in the same solvent mixture. This preparative TLC gave complete separation of cytochalasin C (XXXIV) (9 mg), *Rf* 0.43 (see later), dihydro-cytochalasin D (XXX) (25 mg), *Rf* 0.37, tetrahydro-cytochalasin D[4] (XXIX) (30 mg) *Rf* 0.31, and the starting material I (22 mg), *Rf* 0.23. Dihydro-cytochalasin D (XXX) was obtained as colorless needles (from acetone–*n*-hexane), mp 253—259°, M^+ 509, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1740, 1702, 1008, 968, which has not a bond at 910 cm^{-1} corresponding to the exocyclic double bond at C-5 in I. *Anal.* Calcd. for $C_{30}H_{39}O_6N$: C, 70.70; H, 7.71; O, 18.84; N, 2.75. Found: C, 70.54; H, 7.64; O, 18.38; N, 2.61.

Tetrahydro-cytochalasin C (XXXI) and Dihydro-cytochalasin C (XXXII and XXXIII)——A solution of cytochalasin C (XXXIV) (70 mg) in EtOH (85 ml) was hydrogenated over 10% palladized charcoal (350 mg) for 12 hr at room temperature. The reaction product was crystallized from acetone–*n*-hexane to give a crystalline product (41 mg). This was applied on a thin-layer plate of silica gel and developed with a solvent system of benzene–MeOH (15: 1). The plate was developed four times in the same solvent mixture. This preparative TLC gave complete separation of dihydro-cytochalasin C (XXXII) (26 mg), *Rf* 0.31, its isomer (XXXIII) (6.5 mg), *Rf* 0.25, and tetrahydro-cytochalasin C (XXXI) (3.0 mg), *Rf* 0.21. Dihydro-cytochalasin C (XXXII) was obtained as colorless needles (from *n*-hexane–acetone), mp 290—301° (decomp.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1734, 1705, 1031, 1007, 994, NMR ($CDCl_3$) τ: 8.87 (CH_3, d, J=6.0 Hz), 8.61 (CH_3, s), 8.62 (vinyl CH_3), 8.34 (vinyl CH_3), 7.82 ($OCOCH_3$), which may be identical with dihydro-cytochalasin C[10] obtained by the I.C.I. group. *Anal.* Calcd. for $C_{30}H_{39}O_6N$: C, 70.70; H, 7.71; O, 18.84; N, 2.75. Found: C, 71.06; H, 7.92; O, 18.64; N, 2.84. Its isomer (XXXIII) was obtained as colorless needles (from *n*-hexane–acetone),

mp 245—249°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1737, 1700, 1030, 1018, 1005. *Anal.* Found: C, 70.94; H, 7.70; O, 18.76; N, 2.86. Tetrahydro-cytochalasin C (XXXI) was obtained as colorless needles (from acetone), mp 270—284° (decomp.), M$^+$ 511, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1735, 1700, 1030, 1008, 990.

Cytochalasin C (XXXIV) and XXXV——A solution of cytochalasin D (I) (1 g) in EtOH (250 ml) was hydrogenated over 10% palladized charcoal (5 g) for 30 min at room temperature. The catalyst was filtered off and the filtrate was evaporated to leave a crystalline residue, which was washed with benzene (50 ml). The residue (820 mg) was crystallized from $CHCl_3$–MeOH and recrystallized from acetone to give colorless needles (679 mg, 67.9% yield), mp 261.5—267° (decomp.), $[\alpha]_D^{28}$ −14.7° (±0.7°) (c=0.822 in dioxan), M$^+$ 507, IR ν_{max}^{Nujol} cm^{-1}: 3390, 3175, 3105, 1735, 1700, 1037, 1005, 962, NMR (C_6D_5N) τ: 8.95 (CH_3, d, J=6.0 Hz), 8.62 (vinyl CH_3), 8.45 (CH_3, s), 8.10 (vinyl CH_3), 7.66 ($OCOCH_3$) (*Anal.* Calcd. for $C_{30}H_{37}O_6N$: C, 70.98; H, 7.35; O, 18.91; N, 2.76. Found: C, 71.15; H, 7.09; O, 18.60; N, 2.86), which is identical with cytochalasin C[10] (XXXIV) by comparison of IR and NMR. The benzene-washing was evaporated to leave a residue (169 mg), which was purified by preparative TLC on silica gel to give a 6-oxo-derivative (XXXV) as a colorless powder (120 mg, 12% yield), mp 129—142°, M$^+$ 507, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1740, 1720, 1707. *Anal.* Calcd. for $C_{30}H_{37}O_6N$: C, 70.98; H, 7.35; N, 2.76. Found: C, 71.40; H, 7.16; N, 2.48.

XXXVI.[5] **XXXVII**——Sodium periodate (44 mg) was added to a solution of XII (70 mg) in MeOH (16 ml) and water (4 ml) and heated under reflux for 1 hr. The solution was evaporated and the residue was extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4), and evaporated, leaving a viscous oil (71 mg). The residue was separated by preparative TLC on silica gel to give the starting material XII (23 mg), a keto-aldehyde (XXIV)[4] (15 mg), and XXXVII (10 mg), a viscous oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3395, 1748, 1695, 1108 (OCH_3), 1085—1050, 1020, 976, 940, 913, which is assumed to be C-11-(XXXVII) or C-14-addition product of MeOH to an α,β-unsaturated ketone obtained by aldol condensation of the aldehyde group to the methyl-keto-group in XXIV.