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Viscometric and Electron Microscopic Analysis of Effects of Griseofulvin and its Derivatives on *in vitro* Polymerization of Microtubule Proteins and Depolymerization of Microtubules*

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Griseofulvin is an antibiotic isolated from *Penicillium griseofulvum* DICK and has been reported as "curling factor" since 1946. It is extensively used in the treatment of superficial fungal infections of man and animals, and further it has been shown to block mitosis. Recent many studies indicate that the mode of action of griseofulvin concerns with microtubule proteins and microtubules.

In a preceding paper of this series, we have presented the viscometric and electron microscopic studies of microtubule proteins and microtubules in the presence of griseofulvin and its seventeen derivatives. This paper describes the further studies of (+)-griseofulvin (**1**) and its sixteen derivatives, especially of eight enantiometric pairs, onmicrotubule proteins and microtubules by the same methods described previously.

The structures of seventeen griseofulvin derivatives are shown in Fig. 1. The effects of the derivatives on microtubule proteins and microtubules *in vitro* were calculated as described in a previous paper. Of their activities, the relative strength of the derivatives both upon inhibitory effects on polymerization of microtubule proteins and upon effects on depolymerization of microtubules were proved to show almost the same relations in their orders on the activities. On the other hand, the activities of aggregate formation exhibit a similar tendency with that of their inhibitory activities for polymerization of microtubule proteins. However, the activities of the griseofulvin derivatives were considerably lower than that of natural product, (+)-griseofulvin, indicating that the aggregate formation activity at 4°C is unique for (+)-griseofulvin.

In this communication, accordingly, the structure activity relationship of the inhibitory activities for polymerization of microtubule proteins was investigated. Figs. 2 to 4 represent the dose-responce curves of the inhibition of the polymerization of microtubule proteins. Of the test compounds, (+)-griseofulvin (**1**) was the strongest in the activities at a higher concentration than 25 μM.

In the series of compounds, (+)-griseofulvin (**1**) and (−)-griseofulvin (**5**), and (+)-2′-demethoxygriseofulvin (**2**) and (−)-2′-demethoxygriseofulvin (**6**) are related to

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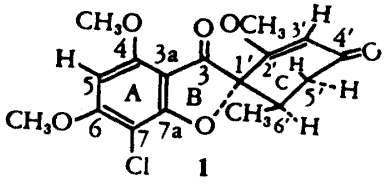


1

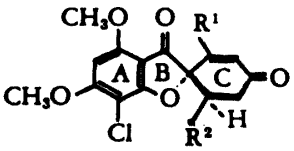
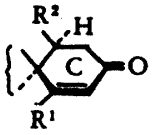
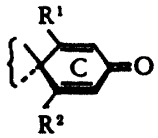
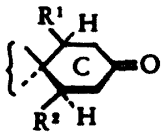
Structure	Compd.	Substituents R¹	Substituents R²
 1–4	1	CH_3O	CH_3
	2	H	CH_3
	3	CH_3	CH_3O
	4	CH_3	H
 5–8	5	CH_3O	CH_3
	6	H	CH_3
	7	CH_3	CH_3O
	8	CH_3	H
 9–14	9	CH_3O	CH_3
	10	$CH_3CH_2CH_2O$	CH_3
	11	H	CH_3
	12	CH_3	CH_3O
	13	CH_3	$CH_3CH_2CH_2O$
	14	CH_3	H
 15–17	15	CH_3O	CH_3
	16	CH_3	CH_3O
	17	H	CH_3

Fig. 1. The Structures of Griseofulvin and Its Derivatives
The numbering of the carbon atoms substituted with R^1 or R^2: (1) when R^1 or R^2 is a methoxy, propox y or H, the carbon number is 2′, and (2) when R^1 or R^2 is a methyl, the carbon number is 6′.

enantiomeric pairs, respectively. The comparison of activities of these enantiomers indicates that the natural series of enantiomers, **1** and **2**, are more active than the corresponding enantiomers, **5** and **6**, respectively. In the compounds from **1** to **8**, the derivatives with a methoxy group at 2′-position showed as a general tendency higher activities than the corresponding derivatives with no methoxy group. The four isomers, **2**, **4**, **6**, and **8**, are different in posiitons of a methyl group and a double bond, and the result of comparison of **2** and **8** shows that the enantiomer (**2**) having a methyl

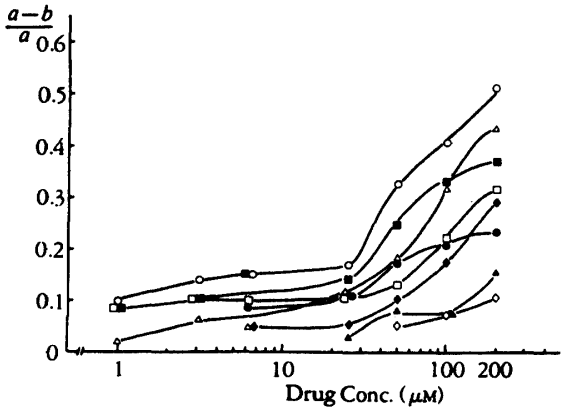


Fig. 2. Dose -Responce Curves of the Inhibition of Polymerization of Microtubule Proteins by Griseofulvin (**1**) a nd Its Seven Derivatives (**2** to **8**) **1** (○), **2**(△), **3**(□), **4**(◇), **5** (●), **6**(▲), **7**(■), and **8**(◆).

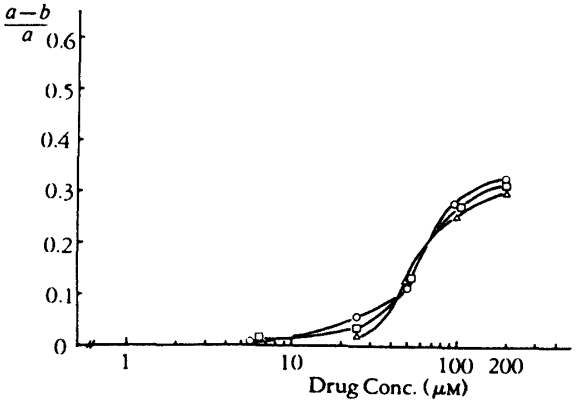


Fig. 3. Dose-Responce Curves of the Inhibtion of Polymerization of Microtubule Proteins by Dihydrogriseofulvin Derivatives (**15** to **17**) **15**(○), **16**(□), and **17**(△).

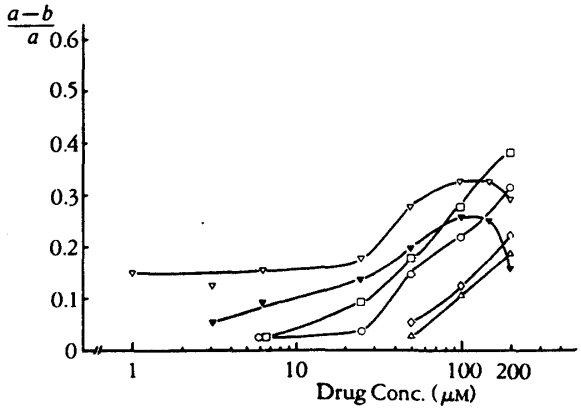


Fig. 4. Dose-Responce Curves of the Inhibition of Polymerization of Microtubule Proteins by Six Dehydrogriseofulvin Derivatives (**9** to **14**) **9**(○), **10**(▽), **11**(△), **12**(□), **13** (▲), and **14** (◇).

group and a double bond at the same positions as natural (+)-griseofulvin (**1**) shows higher activities than those of 8. However, comparison between the isomers of a methyl group position, **6** and 8, shows that **6** having a methyl group at the opposite side with **1** exhibits lower activities than those of 8, and further comparison between the compounds **3** and **5** with a methoxy group and the compounds **4** and **6** with no methoxy group indicates that the former (**3** and **5**) is more active than the latter (**4** and **6**).

In a series of dehydrogriseofulvin derivatives (**9** to **14**), (−)-dehydrogriseofulvin (**9**) and (+)-2′-demethoxydehydrogriseofulvin (**11**) were lower active than their corresponding dihydro-derivatives (**1** and **2**), respectively, and showed almosy same activities with **7**

and 8. However, the corresponding enantiomers (**12** and **14**) of **9** and **11** showed higher activities than those of their corresponding dihydro-derivatives (**3** and **4**), respectively.

Further, the pairs of both **9** and **12** and **11** and **14** have relation to enantiomeric structures and the enantiomers, **12** and **14**, with substituent(s) at opposite site(s) from **1** show higher activities than those of their corresponding enantiomers (**9** and **11**). The compounds : **15**, **16** and **17**, with no double bond on C ring showed almost same inhibitory activities on polymerization of microtubule proteins, and their dose responce curves are shown Fig. 3. 2′-Propoxy derivatives (**10** and **13**) showed the different activities on viscometric analysis as compared with the data described above and their effects on the viscosities at polymerization of microtubule proteins have the peaks at a concentration of ca. 100 μM (Fig. 4). At a concentration of 200 μM, the addition of 2′-propoxy analogs, **10** and **13**, of (−)- and (+)-dehydrogriseofulvin showed higher values on viscosity than that of (+)-griseofulvin (**1**), suggesting the presence of many microtubules. However, no microtubule was observed in the electron microscopic examinations (data not shown).

On the other hand, when incubated at 37°C, microtubule proteins in the presence of 50 μM (+)-griseofulvin (**1**) formed assembled microtubules like a network as observed in an electron micrograph. However, in the presence of the same concentration of **10** or **13**, lesser amounts of microtubules were observed in an electron microscopy although the protein solutions showed higher viscosities than that in the presence of **1**. Moreover, the electron microscopy suggested that the microtubule fibers are covered with aggregated particles and sometimes twisted. The microtubule fibers which were covered with small particles were observed especially in the presence of **10** or **13** although the same phenomena were sometimes observed in the presence of **1** at lower concentrations than 100 μM. The studies described here indicate that, with respect to the C ring moiety of griseofulvin, natural (+)-griseofulvin structure is the most advisable to show its function on microtubule proteins : that is ; a position and numbers of double bond(s), methyl-, and/or methoxy-group(s). And, the aggregated formation activity of **1** is evident, and (+)-2′-demethoxygriseofulvin (**2**) was second although its activity was considerably low. Further, it was suggested that as discussed above **10** and **13** have different types of inhibitory effects on polymerization of microtubule proteins.

Above results were obtained in the experiments that the test samples were added in a solution of microtubule proteins just after starting the incubation at 37°C from 4°C. On the other hand, in order to find the effect of preincubation at low temperature, the inhibition of polymerization of microtubule proteins was examined by an elevation of the temperature to 37°C after being incubated of the solution of microtubule proteins at 4°C for 30 min after adding the test samples. Preincubation of microtubule proteins with griseofulvin derivatives resulted in the elevation of activities from a point of views of viscosity with some exceptions as observed in the derivatives, **2**, 8 and **10**. Further

experiments are necessary to clarify the structure activity relations of these derivatives on microtubule proteins and microtubules.