Syntheses and Antifungal Activity of *dl*-Griseofulvin and Its Congeners. II[1)]

Yasuo TAKEUCHI,*,[a] Hideo TOMOZANE,[a] Keiji MISUMI,[a] Kuniko YATA,[a] Teruyuki KAWATA,[a] Yoshiko NIINO,[a] Masatoshi YAMATO,[b] and Takashi HARAYAMA[a]

Faculty of Pharmaceutical Sciences, Okayama University,[a] Tsushima-naka 1–1–1, Okayama 700, Japan and Faculty of Science, Okayama University of Science,[b] Ridaicho, Okayama 700, Japan.

Received September 4, 1996; accepted October 14, 1996

Griseofulvin derivatives, *dl*-6′-demethyl-6′-ethylgriseofulvin (*dl*-5) and *dl*-6′-demethyl-6′-phenylgriseofulvin (*dl*-6) were prepared by application of a synthetic method developed by us. Antifungal activity of these derivatives decreased in the order of *dl*-griseofulvin (*dl*-1) > *dl*-5 ≫ *dl*-6 (inactive). The reaction of these derivatives with ethanethiol gave two types of compounds, 2′-(ethylthio)griseofulvin (15) and 4′-(ethylthio)isogriseofulvin (16). The relationship between the ratios of isolated yield of 15 and 16 and antifungal the activity of griseofulvin derivatives is discussed.

Key words griseofulvin derivative; thiogriseofulvin; epigriseofulvin; isogriseofulvin; antifungal activity; thiol

d-Epigriseofulvin (*d*-**2**), the diastereomeric isomer of the antifungal agent *d*-griseofulvin (*d*-**1**), lacks the activity, and the racemate, *dl*-griseofulvin (*dl*-**1**), has half the antifungal activity of *d*-**1**.[2)] These facts show that the steric environment around positions 1′ and 6′ of griseofulvin is important for the activity. We have continued to study the structure–activity relationship of 6′-substituted griseofulvin derivatives and found that the *dl*-6′-demethyl derivative (*dl*-**3**) and *dl*-6′-methyl derivative (*dl*-**4**) have decreased antifungal activities in comparison with *dl*-**1**.[1)] The focus of our study was derivatives in which the methyl group at position 6′ of *dl*-**1** is replaced. For this purpose, our new synthetic method was expected to be of use.[3)] Also, it is known that *d*-griseofulvin (*d*-**1**) reacts with thiol to give various thiogriseofulvin derivatives.[4)] In order to investigate this in detail, we examined the reaction of *d*-**1** or its derivatives with SH compounds. In this paper, we describe the synthesis and antifungal activity of *dl*-6′-demethyl-6′-ethylgriseofluvin (*dl*-**5**) and *dl*-6′-demethyl-6′-phenylgriseofluvin (*dl*-**6**), and the reaction of *d*-**1**, *d*-**2**, *dl*-**5**, and *dl*-**6** with ethanethiol.

Synthesis *dl*-Ethyl (*dl*-**5**) and *dl*-phenyl (*dl*-**6**) derivatives could be synthesized by the application of our method, as shown in Chart 1. β-Alkoxyketones, (**9a—c**) were prepared from trimethylsilylenol ether (**7**) and acetals (**8a—c**) by use of the Mukaiyama–Michael reaction.[5)] Condensation of benzofuranone (**10**)[6)] and mono-sulfinyl ketones, which were prepared from **9a—c** by oxidation with *m*-chloroperbenzoic acid (*m*-CPBA), gave the *exo* olefin compounds (**11a—c**) with migration of the double bond. We could not determine the configuration of the

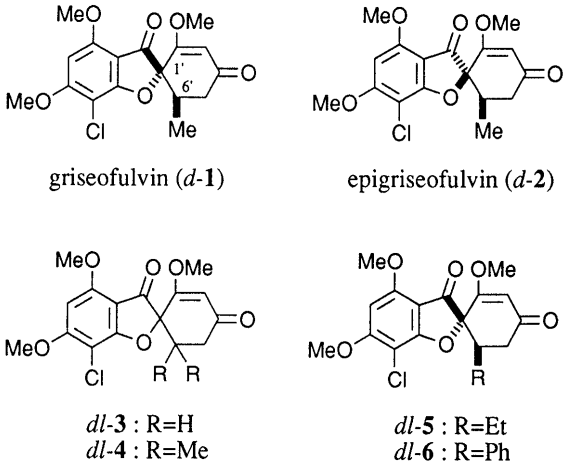

Fig. 1. Griseofulvin Derivatives

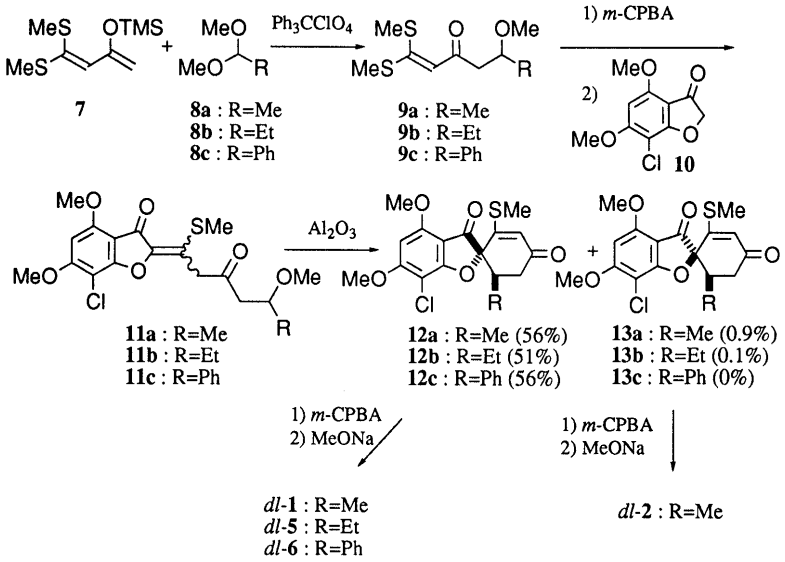

Chart 1

* To whom correspondence should be addressed.

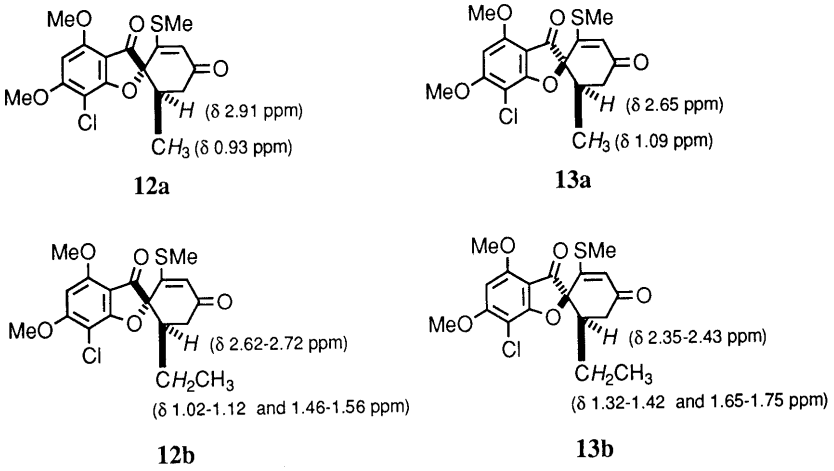

Fig. 2. ^{1}H-NMR (500 MHz) Data for Thiogriseofulvins (**12a, b, 13a, b**)

Table 1. Antifungal Activities of Griseofulvin Derivatives (*in Vitro*)

Compound	*MIC* (mg/ml)	
	T.m. T-14[a]	*T.m.* T-16[b]
d-**1** (*d*-griseofulvin)	3.13	1.56
dl-**1** (*dl*-griseofulvin)	6.25	3.13
dl-**2** (*dl*-epigriseofulvin)	>100	>100
dl-**5**	12.5	6.25
dl-**6**	>100	>100

a) *T.m.* T-14 = *Trichophyton mentagrophytes* T-14. b) *T.m.* T-16 = *Trichophyton mentagrophytes* T-16.

exo olefin. The ring-closure reaction of **11** could be achieved with high diastereoselectivity by alumina treatment to give **12** as the major product. In particular, the reaction of **11c** with alumina gave only **12c**. *dl*-Griseofulvin derivatives (*dl*-**1**, *dl*-**5**, and *dl*-**6**) were obtained successfully by oxidation of the 2′-thiogriseofulvin derivatives (**12**) with *m*-CPBA, followed by addition-elimination reaction with sodium methoxide.

We determined the structures of **12b** and **13b** from the ^{1}H-NMR data in comparison with those of the thiogriseofulvin derivatives, **12a** and **13a**, whose structures were elucidated by conversion to known compounds, *dl*-griseofulvin (*dl*-**1**) and *dl*-epigriseofulvin (*dl*-**2**),[7] respectively. In the ^{1}H-NMR spectra of **12a** and **13a**, differences were observed in the chemical shifts of the 6′ proton and 6′ methyl or ethyl protons, as shown in Fig. 2. The 6′ methine proton of **12a** was observed at lower field than that of **13a**. On the other hand, the methyl protons at the 6′ position of **12a** were observed at higher field than those of **13a**. Similar results were obtained for **12a** and **13a**. We could not determine the structure of **12c** because **13c** could not be isolated for comparison. However, we think that the relative configuration of **12c** is the same as that of **12a** or **12b**, because **12c** was obtained under the same reaction conditions as **12a** and **12b**.

Antifungal Activity The antifungal activity of griseofulvin derivatives was examined and the minimum inhibitory concentration (*MIC*) values against *Trichophyton mentagrophytes* are listed in Table 1. The bulkiness of the 6′-substituent of griseofulvin derivatives strongly affected the activity. The antifungal activity of the *dl*-6′-ethyl derivative (*dl*-**5**) exhibited 1/4 value of *dl*-**1**. On the other hand, *dl*-6′-phenyl derivative (*dl*-**6**) lacked the activity. The activities of the 6′-substituted derivatives decreased in order of Me (*dl*-**1**) > Et(*dl*-5) ≫ Ph (*dl*-**6**).

Reactions with Ethanethiol Koe and Celmer reported that the reaction products depended upon the kind of thiol in the reaction of griseofulvin (*d*-**1**) with thiols.[4] We thought that the reaction products might change depending on the kind of griseofulvin derivative.

Treatment of *d*-griseofulvin (*d*-**1**) with ethanethiol in the presence of *p*-toluenesulfonic acid in methylene chloride gave *d*-ethylthiogriseofulvin (*d*-**14a**) in 52% yield and *d*-ethylthioisogriseofulvin (*d*-**15a**) in 27% yield. Under the same conditions, **14b** (30% yield) and **15b** (24% yield) were obtained from the *dl*-6′-ethyl derivative (*dl*-**5**), and **14c** (12% yield) and **15c** (48% yield) from the *dl*-6′-phenyl derivative (*dl*-**6**). The structures of the 2′-ethylthio derivatives (*d*-**14a** and **14b**) and 4′-ethylthio derivatives (**15a—c**) were confirmed by transformation to the corresponding starting materials (*d*-**1** and *dl*-**5**) and *d*-isogriseofulvin (*d*-**16a**),[8] **16b**, and **16c**, respectively (Chart 2).

The position of the ethylthio group of the major product obtained from the reaction of *d*-epigriseofulvin (*d*-**2**)[9] with ethanethiol was different from that of *d*-**1** with ethanethiol. Treatment of *d*-**2** with ethanethiol in the presence of *p*-toluenesulfonic acid in methylene chloride gave mainly *d*-4′-ethylthioepiisogriseofulvin (*d*-**15d**) in 43% yield, with l-2′-ethylthioepigriseofulvin (*l*-**14d**) in 14% yield, as shown in Chart 3. The structure of each product confirmed by transformation to the known product *d*-**2** or *d*-**16d**,[9] respectively.

Results and Discussion

Our new synthetic method for *dl*-griseofulvin was useful for the synthesis of 6′-substituted griseofulvin derivatives.

The activities of 6′-substituted derivatives decreased in order of Me (*d*-**1**) > Me (*dl*-**1**) > Et (*dl*-**5**) ≫ Ph (*dl*-**6**) = epi Me (*d*-**2**). Thus, the steric factor of the 6′-substituent is important role for the activity and the methyl group is the best substituent in this respect. The reaction of the 6′-derivatives with ethanethiol gave two products, the 2′- and 4′-ethylthio derivatives. The ratios of isolated yield of 2′-ethylthio derivatives against 4′-ethylthio derivatives

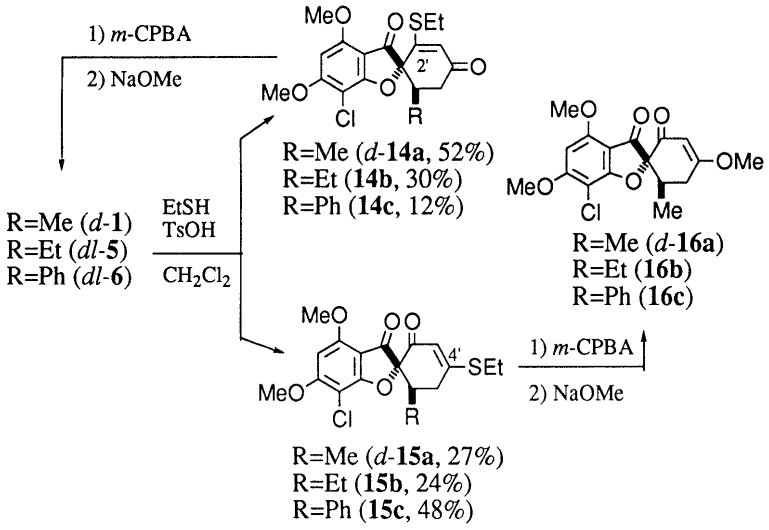

Chart 2

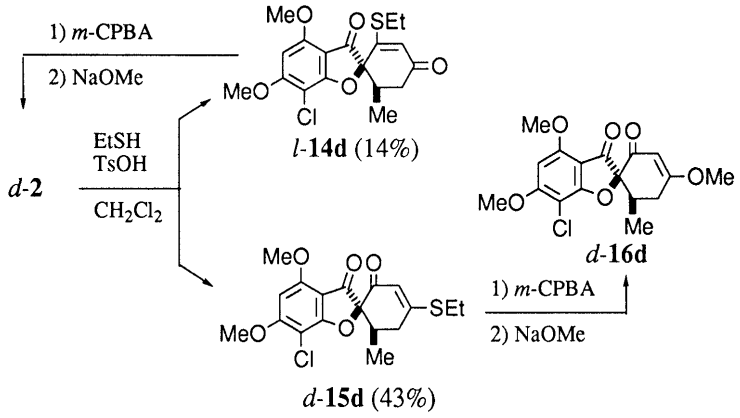

Chart 3

in the reaction decreased in order of Me (*dl*-**1**) > Et (*dl*-**5**) ≫ epi Me (*d*-**2**) = Ph (*dl*-**6**).

We could not explain the similarity of the order of antifungal activity to that of the product ratio in the reaction of these derivatives with ethanethiol. However, considering the fact that griseofulvin binds *in vivo* with microtubulin,[10] we speculate that binding between griseofulvin and an SH group may play an important role in manifestation of the antifungal activity.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. Mass spectra (MS) were recorded on a VG-70SE spectrometer. ^{1}H-NMR spectra were run on a Hitachi R-1500 (60 MHz) or a Varian VXR-500 (500 MHz) spectrometer. Optical rotations were measured on a JASCO DIP-4 spectrometer. Analytical HPLC was performed on Chemcosorb 5Si-U (Chemco). Merck Silica gel 60 (230—400 mesh) was employed for column chromatography. Extracts were dried over anhydrous $MgSO_4$.

5-Methoxy-1,1-bis(methylthio)-1-hepten-3-one (9b) Under an Ar atmosphere, **8b** (11.0 ml, 89.6 mmol) was added dropwise at 0 °C to a solution of **7** (10.0 g, 42.6 mmol) in dry CH_2Cl_2 (21.6 ml). Ph_3CClO_4 (0.72 g, 2.14 mmol) was added portionwise at 0 °C, and the reaction mixture was stirred at the same temperature for 50 min, then poured into ice water (400 ml), and 10% aqueous $NaHCO_3$ (100 ml) was added. The whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The residue was purified by distillation to give **9b** (6.11 g, 61%) as a slightly green oil, bp 120—130 °C/0.2 mmHg. IR (Nujol) ν: 1640 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 0.92 (3H, t, J = 7.0 Hz, $CH_2C\underline{H}_3$), 1.30—1.80 (2H, m, $C\underline{H}_2CH_3$), 2.45, 2.47 (each 3H, each s, each SCH_3), 2.30—2.82 (2H, m, $COCH_2$), 3.34 (3H, s, OCH_3), 3.57 (1H, sextet, J = 5.7 Hz, $CH_3OC\underline{H}$), 6.08 (1H, s, C=C$\underline{H}$). FAB-MS (positive ion mode) m/z: 235 [(M + 1)$^+$].

5-Methoxy-1,1-bis(methylthio)-6-phenyl-1-hexen-3-one (9c) Under an Ar atmosphere, $TrClO_4$ (0.3 g, 0.9 mmol) was added portionwise at 0 °C to a solution of **8c** (3.0 g, 18 mmol), and **7** (2.0 g, 8.9 mmol) in dry CH_2Cl_2 (2 ml). The reaction mixture was stirred at the same temperature for 15 min, then aqueous $NaHCO_3$ (10%, 100 ml) was added, and the whole was extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt:hexane = 1 : 9) to give **9c** (1.2 g, 45%) as an oil. IR (Nujol) ν: 1640 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.31, 2.34 (each 3H, each s, each $SC\underline{H}_3$), 2.59—2.96 (2H, m, $COCH_2$), 3.16 (3H, s, OCH_3), 4.67 (1H, t, J = 5.4 Hz, $CH_3OC\underline{H}$), 5.94 (1H, s, C=CH), 7.26 (5H, s, C_5-H). FAB-MS (positive ion mode) m/z: 283 [(M + 1)$^+$].

7-Chloro-4,6-dimethoxy-2-[5-methoxy-1-(methylthio)-3-oxoheptylidene]-3(2*H*)-benzofuranone (11b) *m*-Chloroperbenzoic acid (5.85 g, 33.9 mmol) was added portionwise at 0 °C to a solution of **9b** (5.3 g, 22.6 mmol) in dry CH_2Cl_2 (500 ml). The reaction mixture was stirred at the same temperature for 15 min and 10% aqueous Na_2SO_3 was added, then the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with 10% aqueous $NaHCO_3$ and saturated NaCl solution, dried, and evaporated *in vacuo*. Potassium *tert*-butoxide (2.77 g, 24.6 mmol) was added portionwise at 0 °C to a mixture of the residue (about 5.1 g) and dry tetrahydrofuran (THF) (180 ml). The whole was stirred at the same temperature for 20 min. Benzofuranone **10**[6] (5.61 g, 22.4 mmol) dissolved in dry THF (40 ml) was added at 0 °C. The reaction mixture was stirred at the same temperature for 20 min, then poured into ice water (600 ml), and 10% aqueous HCl solution (150 ml) was added. The whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water,

dried, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt : hexane = 1 : 1) and recrystallization (CH_2Cl_2 and petr. ether) to give **11b** (6.13 g, 68%) as yellow prisms, mp 139—141 °C. IR (Nujol) *v*: 1715, 1680 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.89 (3H, t, J=7.4 Hz, $CH_2C\underline{H}_3$), 1.50—1.60 (2H, m, $C\underline{H}_2CH_3$), 2.53 (3H, s, SCH_3), 2.65 (1H, dd, J=16.1, 4.5 Hz, COCH$\underline{H}$), 2.80 (1H, dd, J=16.1, 7.8 Hz, COC$\underline{H}$H), 3.32 (3H, s, OCH_3), 3.64—3.71 (1H, m, C$\underline{H}$$OCH_3$), 3.96, 3.99 (each 3H, each s, each OCH_3), 4.14, 4.34 (each 1H, each d, each J=7.2 Hz, COC$\underline{H}\underline{H}$), 6.15 (1H, s, C_5-H). FAB-MS (positive ion mode) *m/z*: 417 [$(M+1)^+ + 2$], 415 [$(M+1)^+$]. *Anal.* Calcd for $C_{19}H_{23}ClO_6S$: C, 55.00; H, 5.59. Found: C, 55.26; H, 5.32.

7-Chloro-4,6-dimethoxy-2-[5-methoxy-1-(methylthio)-3-oxo-5-phenylpentylidene]-3(2*H*)-benzofuranone (11c) *m*-Chloroperbenzoic acid (0.23 g, 1.3 mmol) was added portionwise at 0 °C to a solution of **9c** (0.27 g, 0.94 mmol) in dry CH_2Cl_2 (25 ml). The reaction mixture was stirred at the same temperature for 30 min, 10% aqueous $NaHCO_3$ was added, and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with 10% aqueous Na_2SO_3 and water, dried, and evaporated *in vacuo*. *tert*-BuOK (0.11 g, 0.94 mmol) was added portionwise at 0 °C to a solution of the residue (about 0.18 g) in dry THF (5 ml). Benzofuranone **10**[6] (0.24 g, 0.94 mmol) dissolved in dry THF (3 ml) was added at 0 °C, and the reaction mixture was stirred at the same temperature for 15 min, then acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt : hexane = 1 : 1) and recrystallization (AcOEt and hexane) to give **11c** (0.23 g, 64%) , mp 143—145 °C. IR (Nujol) *v*: 1710, 1675 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.46 (3H, s, SCH_3), 2.54—3.13 (2H, m, $COCH_2$), 3.20 (3H, s, OCH_3), 3.98 (6H, s, $OCH_3 \times 2$), 4.07—4.54 (2H, m, $CHCH_2$), 4.55—4.88 (1H, m, $CH_3OC\underline{H}$), 6.18 (1H, s, C5-$\underline{H}$), 7.36 (5H, s, Ar-H). FAB-MS (positive ion mode) *m/z*: 465 [$(M+1)^+ + 2$], 463 [$(M+1)^+$]. *Anal.* Calcd for $C_{23}H_{23}ClO_6S$: C, 59.67; H, 5.01. Found: C, 59.48; H, 4.86.

***dl*-2′-Demethoxy-2′-(methylthio)griseofulvin (12a) and *dl*-2′-Demethoxy-2′-(methylthio)epigriseofulvin (13a)** Under an Ar atmosphere, a mixture of **11a** (5.42 g, 13.5 mmol), Al_2O_3 (for column chromatography, 3.22 g, 31.6 mmol), and dry THF (347 ml) was stirred under reflux for 10 h, then filtered and the filtrate was evaporated *in vacuo*. The ratio (7 : 1) of **12a** and **13a** in the residue was determined by HPLC (column, Chemcosorb 5Si-U; column temperature, room temperature; eluent, CH_2Cl_2 : MeOH = 150 : 1; flow rate, 1.0 ml/min; wavelength, 254 nm). Recrystallization (CH_2Cl_2 and Et_2O) of the residue gave **12a** (4.69 g, 56%) as colorless prisms, mp 239—242 °C (lit.[3a] 234—246 °C). IR (Nujol) *v*: 1710, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.93 (3H, d, J=6.6 Hz, C6′-C$\underline{H}_3$), 2.21 (3H, s, SCH_3), 2.44 (1H, dd, J=17.1, 4.9 Hz, C5′$\underline{H}$H), 2.91 (1H, dqd, J=13.7, 6.6, 4.9 Hz, C6′-$\underline{H}$), 3.04 (1H, dd, J=17.1, 13.7 Hz, C5′-H$\underline{H}$), 3.97, 4.03 (each 3H, each s, each OCH_3), 5.91 (1H, s, C3′-$\underline{H}$), 6.14 (1H, s, C5-$\underline{H}$). EI-MS *m/z*: 370 [$(M+2)^+$], 368 [M^+].

The filtrate of recrystallization was evaporated and the residue was purified by preparative TLC (CH_2Cl_2) and recrystallization (CH_2Cl_2 and Et_2O) to give **13a** (43 mg, 0.9%) as colorless needles, mp 202—205 °C. IR (Nujol) *v*: 1715, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.09 (3H, d, J=6.7 Hz, C6′-CH_3), 2.26 (3H, s, SCH_3), 2.48 (1H, dd, J=17.0, 7.5 Hz, C5′$\underline{H}$H), 2.65 (1H, dqd, J=7.5, 6.7, 4.9 Hz, C6′-H), 2.94 (1H, dd, J=17.0, 4.9 Hz, C5′-H$\underline{H}$), 3.99, 4.03 (each 3H, each s each $OC\underline{H}_3$), 5.96 (1H, s, C3′-H), 6.14 (1H, s, C5-H). EI-MS *m/z*: 370 [$(M+2)^+$], 368 [M^+]. *Anal.* Calcd for $C_{17}H_{17}ClO_5S$: C, 55.36; H, 4.65. Found: C, 55.47; H, 4.75.

***dl*-2′-Demethoxy-6′-demethyl-6′-ethyl-2′-(methylthio)griseofulvin (12b) and *dl*-2′-Demethoxy-6′-demethyl-6′-ethyl-2′-(methylthio)epigriseofulvin (13b)** Under an Ar atmosphere, a mixture of **11b** (1.13 g, 3.16 mmol), Al_2O_3 (for column chromatography, 3.22 g, 31.6 mmol), and dry THF (84 ml) was stirred under reflux for 10 h, then filtered, and the filtrate was evaporated *in vacuo*. The ratio (10 : 1) of **12b** and **13b** in the residue was determined by HPLC (column, Chemcosorb 5Si-U; column temperature, room temperature; eluent, CH_2Cl_2 : MeOH = 150 : 1; flow rate, 1.0 ml/min; wavelength, 254 nm). Recrystallization (CH_2Cl_2 and Et_2O) of the residue gave **12b** (0.89 g, 51%) as colorless plates, mp 206—207 °C. IR (Nujol) *v*: 1710, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, t, J=7.4 Hz, $CH_2C\underline{H}_3$), 1.02—1.12, 1.47—1.56 (each 1H, each m, C$\underline{H}$HCH$_3$), 2.21 (3H, s, $SC\underline{H}_3$), 2.61 (1H, dd, J=16.7, 5.0 Hz, C5′-H$\underline{H}$), 2.62—2.72 (1H, m, C6′-H). 2.92 (1H, dd, J=16.7, 12.9 Hz, C5′-$\underline{H}$H), 3.91, 4.03 (each 3H, each s, each $OC\underline{H}_3$), 5.92 (1H, s, C3′-H), 6.14 (1H, s, C5-H). FAB-MS (positive ion mode) *m/z*: 385 [$(M+1)^+ + 2$], 383 [$(M+1)^+$]. *Anal.* Calcd for $C_{18}H_{19}ClO_5S$: C, 56.47; H, 5.00. Found: C, 56.20; H, 5.02.

The filtrate of recrystallization was evaporated and the residue was purified by column chromatography (AcOEt : hexane = 3 : 1) and recrystallization (CH_2Cl_2 and Et_2O) to give **13b** (1.2 mg, 0.1%) as colorless prisms, mp 204—205 °C. IR (Nujol) *v*: 1710, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.91 (3H, t, J=7.5 Hz, $CH_2C\underline{H}_3$), 1.32—1.42, 1.65—1.75 (each 1H, each m, C$\underline{H}\underline{H}CH_3$), 2.25 (3H, s, SCH_3), 2.35—2.43 (1H, m, C6′-H), 2.53 (1H, dd, J=17.2, 4.9 Hz, C5′-H$\underline{H}$), 3.03 (1H, dd, J=17.2, 4.9 Hz, C5′-$\underline{H}$H), 4.00, 4.03 (each 3H, each s, each OCH_3), 5.97 (1H, s, C3′-H), 6.17 (1H, s, C5-$\underline{H}$). EI-MS *m/z*: 384 [$(M+2)^+$], 382 [M^+]. *Anal.* Calcd for $C_{18}H_{19}ClO_5S$: C, 56.47; H, 5.00. Found: C, 56.89; H, 5.18.

***dl*-2′-Demethoxy-6′-demethyl-2′-(methylthio)-6′-phenylgriseofulvin (12c)** A mixture of **11c** (0.23 g, 0.50 mmol), Al_2O_3 (for column chromatography, 2.3 g), and dry Et_2O (15 ml) was stirred under reflux for 6 h, then filtered, and filtrate was evaporated *in vacuo*. Recrystallization (CH_2Cl_2 and hexane) of the residue gave **12c** (0.12 g, 56%) as colorless prisms, mp 247—252 °C. IR (Nujol) *v*: 1700, 1660 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.27 (3H, s, SCH_3), 2.56 (1H, dd, J=15.3, 2.7 Hz, C5′-H$\underline{H}$), 3.77—4.03 (2H, m, C5′H$\underline{H}$, C6′-H), 3.85, 3.90 (each 3H, each s, each $OC\underline{H}_3$), 5.89 (1H, s, C3′-H), 6.02 (1H, s, C4-H), 7.04—7.46 (5H, m, Ar-H). FAB-MS (positive ion mode) *m/z*: 433 [$(M+1)^+ + 2$], 431 [$(M+1)^+$]. *Anal.* Calcd for $C_{22}H_{19}ClO_5S$: C, 61.32; H, 4.44. Found: C, 61.03; H, 4.36.

***dl*-6′-Demethyl-6′-ethylgriseofulvin (*dl*-5)** Under an Ar atmosphere, *m*-CPBA (0.87 g, 5.04 mmol) was added to a solution of **12b** (1.29 g, 3.38 mmol) in CH_2Cl_2 (63 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then 10% aqueous Na_2SO_3 (40 ml) was added, and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated NaCl solution, dried, and evaporated *in vacuo*. Under an Ar atmosphere, NaOMe (0.30 M in MeOH, 13.5 ml, 4.05 mmol) was added dropwise to a solution of the residue in dry benzene (14 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and poured into ice water. The whole was acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The residue was purified by column chromatography (Si_2O, AcOEt : hexane = 2 : 1) to give *dl*-**5** (0.60 g, 49%) as colorless needles, mp 209—210 °C (CH_2Cl_2 and Et_2O). IR (Nujol) *v*: 1700, 1670 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, t, J=7.4 Hz, $CH_2C\underline{H}_3$), 1.08—1.20, 1.44—1.55 (each 1H, each m, C$\underline{H}\underline{H}CH_3$), 2.55—2.63 (2H, m, C5′-H$\underline{H}$, C6′-H), 2.90 (1H, dd, J=17.5, 14.5 Hz, C5′-$\underline{H}$H), 3.61 (3H, s, C2′-OCH_3), 3.97, 4.02 (each 3H, each s, each OCH_3), 5.53 (1H, s, C3′-H), 6.12 (1H, s, C5-H). EI-MS *m/z*: 368 [$(M+2)^+$], 366 [M^+]. *Anal.* Calcd for $C_{18}H_{19}ClO_6$: C, 58.94; H, 5.22. Found: C, 59.18; H, 5.33.

***dl*-6′-Demethyl-6′-phenylgriseofulvin (*dl*-6)** Under an Ar atmosphere, *m*-CPBA (0.17 g, 0.99 mmol) was added to a solution of **12c** (0.29 g, 0.67 mmol) in CH_2Cl_2 (10 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then 10% aqueous Na_2SO_3 (40 ml) was added, and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$, and saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. Under an Ar atmosphere, NaOMe (0.30 M in MeOH, 2.7 ml, 0.80 mmol) was added dropwise to a solution of the residue in dry benzene (14 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 5 min. and poured into ice water. The whole was acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, AcOEt : hexane = 2 : 1) to give *dl*-**6** (0.23 g, 83%) as colorless needles, mp 222—226 °C (benzene and hexane). IR (Nujol) *v*: 1705, 1680 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 2.59 (1H, dd, J=14.0, 2.3 Hz, C5′-H$\underline{H}$), 3.39—4.14 (2H, m, C5′-H$\underline{H}$ and C6′-H), 3.66 (3H, s, C2′-OCH_3), 3.83, 3.90 (each 3H, each s, each $OC\underline{H}_3$), 5.63 (1H, s, C3′-$\underline{H}$), 5.89 (1H, s, C5-H), 7.06—7.42 (5H, m, Ar-H). FAB-MS (positive ion mode) *m/z*: 417 [$(M+1)^+ + 2$], 415 [$(M+1)^+$]. *Anal.* Calcd for $C_{22}H_{19}ClO_6$: C, 63.70; H, 4.62. Found: C, 63.82; H, 4.57.

***dl*-Epigriseofulvin (*dl*-2)** Under an Ar atmosphere, *m*-CPBA (20.6 mg, 0.119 mmol) was added to a solution of **13a** (40 mg, 0.108 mmol) in CH_2Cl_2 (2 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, then 5% aqueous Na_2SO_3 (40 ml) was added and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated NaCl solution, dried, and evaporated *in vacuo*.

Under an Ar atmosphere, NaOMe (0.30 M in MeOH, 0.4 ml, 0.12 mmol) was added dropwise to a solution of the residue in dry benzene (0.4 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and poured into ice water. The mixture was acidified with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The residue was purified by preparative TLC (AcOEt : hexane = 2 : 1) to give **2** (17 mg, 43%) as colorless needles, mp 252—254 °C (CH_2Cl_2 and petr. ether) (lit.[7] 250—251 °C).

Reaction of *d*-Griseofulvin (*d*-1) with EtSH Under an Ar atmosphere, EtSH (1.05 ml, 14.2 mmol) was added dropwise to a solution of *d*-**1** (1.00 g, 2.83 mmol) and *p*-TsOH (0.54 g, 2.83 mmol) in dry CH_2Cl_2 (7 ml). The reaction mixture was stirred at room temperature for 40 min, then aqueous NaOCl solution (50 ml) was added and the whole was acidified with 10% aqueous KOH solution and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution, dried, and evaporated. The ratio (2.5 : 1) of *d*-2′-demethoxy-2′-(ethylthio)griseofulvin (*d*-**14a**) and *d*-4′-demethoxy-4′-(ethylthio)isogriseofulvin (*d*-**15b**) in the residue was determined by HPLC (column, Chemcosorb 5Si-U; column temperature, room temperature; eluent, AcOEt : hexane = 1 : 1; flow rate, 2.0 ml/min; wavelength, 254 nm). Separation by column chromatography gave *d*-**14a** (eluent : AcOEt : hexane = 1 : 1.5) and *d*-**15a** (eluent: AcOEt : hexane = 1 : 1). *d*-**14a** (558 mg, 52%), mp 184—185 °C (lit.[4] 180—181 °C). *d*-**15a** (288 mg, 27%), mp 177—178 °C (lit.[4] 173—175 °C).

Reaction of *dl*-6′-Demethyl-6′-ethylgriseofulvin (*dl*-5) with EtSH Under an Ar atmosphere, EtSH (1.20 ml, 16.2 mmol) was added dropwise to a solution of *dl*-**5** (1.19 g, 3.24 mmol) and *p*-TsOH (0.56 g, 3.24 mmol) in dry CH_2Cl_2 (7.6 ml). The reaction mixture was stirred at room temperature for 40 min, then aqueous NaOCl solution (50 ml) was added. The whole was acidified with 10% aqueous KOH solution and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. The ratio (2 : 1) of *dl*-2′-demethoxy-6′-demethyl-6′-ethyl-2′-(ethylthio)griseofulvin (**14b**) and *d*-4′-demethoxy-6′-demethyl-6′-ethyl-4′-(ethylthio)isogriseofulvin (**15b**) in the residue was determined by HPLC (column, Chemcosorb 5Si-U; column temperature, room temperature; eluent, AcOEt : hexane = 1 : 1; flow rate, 1.0 ml/min; wavelength, 254 nm). Separation and purification of the residue by column chromatography gave **15b** (eluent : AcOEt : hexane = 1 : 2) and **14b** (eluent : AcOEt : hexane = 1 : 1).

14b (0.387 g, 30%) as colorless needles, mp 182—184 °C (CH_2Cl_2 and petr. ether). IR (KBr) *v*: 1710, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 7.4 Hz, C6′-CH_2CH_3), 1.00—1.11, 1.46—1.55 (each 1H, each m, C6′-CHHCH$_3$), 1.24 (3H, t, J = 7.4 Hz, SCH_2CH_3), 2.60 (1H, dd, J = 16.7, 5.2 Hz, C5′-HH), 2.61—2.69 (1H, m, C6′-H), 2.68, 2.73 (each 1H, each dq, J = 1.29, 7.4 Hz, SCHHCH$_3$), 2.90 (1H, dd, J = 16.7, 13.0 Hz, C5′-HH), 3.97, 4.03 (each 3H, each s, each OCH_3), 5.95 (1H, s, C3′-H), 6.14 (1H, m, C5-H). EI-MS *m/z*: 398 $[M+2]^+$, 396 $[M]^+$. *Anal.* Calcd for $C_{19}H_{21}ClO_5S$: C, 57.50; H, 5.33. Found: C, 57.23; H, 5.35.

15b (0.310 g, 24%) as colorless needles, mp 192—194 °C (CH_2Cl_2 and petr. ether). IR (KBr) *v*: 1700, 1650 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.91 (3H, t, J = 7.5 Hz, C6′-CH_2CH_3), 1.17—1.30, 1.51—1.64 (each 1H, each m, C6′-CHHCH$_3$), 1.36 (3H, t, J = 7.4 Hz, SCH_2CH_3), 2.58—2.68 (2H, m, C5′-HH and C6′-H), 2.87, 2.89 (each 1H, each dq, each J = 13.1, 7.4 Hz, SCHHCH$_3$), 3.21 (1H, ddd, J = 17.8, 12.3, 1.9 Hz, C5′-HH), 3.92, 4.00 (each 3H, each s, each OCH_3), 5.91 (1H, d, J = 1.9 Hz, C3′-H), 6.08 (1H, m, C5-H). EI-MS *m/z*: 398 $[M+2]^+$, 396 $[M]^+$. *Anal.* Calcd for $C_{19}H_{21}ClO_5S$: C, 57.50; H, 5.33. Found: C, 57.39; H, 5.36.

Reaction of *dl*-6′-Demethyl-6′-phenylgriseofulvin (*dl*-6) with EtSH Under an Ar atmosphere, EtSH (0.54 ml, 7.25 mmol) was added dropwise to a solution of *dl*-**6** (0.60 g, 1.45 mmol) and *p*-TsOH (0.26 g, 1.45 mmol) in dry CH_2Cl_2. The reaction mixture (3 ml) was stirred at room temperature for 2 h, then aqueous NaOCl solution (50 ml) was added, and the whole was acidified with 10% aqueous KOH solution and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution, dried, and evaporated *in vacuo*. Separation and purification of the residue by column chromatography (eluent : AcOEt : hexane = 1 : 1) gave **15c** and **14c**.

14c (0.080 g, 12%) as colorless needles, mp 243—245 °C. IR (Nujol) *v*: 1715, 1660 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.28 (3H, t, J = 6.6 Hz, SCH_2CH_3), 2.36—3.06 (3H, m, C5′-H$_2$, C6′-H), 2.80 (2H, q, J = 6.6 Hz, SCH_2CH_3), 3.87, 3.91 (each 3H, each s, each OCH_3), 5.92 (1H, s, C3′-H), 6.08 (1H, m, C5-H), 6.96-7.41 (5H, m, Ar-H). FAB-MS (positive ion mode) *m/z*: 447 $[(M+1)^+ + 2]$, 445 $[(M+1)^+]$. *Anal.* Calcd for $C_{23}H_{21}ClO_5S$: C, 62.09; H, 4.76. Found: C, 61.58; H, 4.81.

15c (0.310 g, 48%) as colorless needles, mp 211—212 °C. IR (Nujol) *v*: 1705, 1655 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.35 (3H, t, J = 6.6 Hz, SCH_2CH_3), 2.50—3.40 (3H, m, C5′-H$_2$, C6′-H), 2.93 (2H, q, J = 6.6 Hz, SCH_2CH_3), 3.73, 3.82 (each 3H, each s, each OCH_3), 5.84 (1H, s, C3′-H), 5.98 (1H, m, C5-H), 6.96—7.43 (5H, m, Ar-H). FAB-MS (positive ion mode) *m/z*: 447 $[(M+1)+2]$, 445 $[(M+1)^+]$. *Anal.* Calcd for $C_{23}H_{21}ClO_5S$: C, 62.09; H, 4.76. Found: C, 61.63; H, 4.83.

Reaction of *d*-Epigriseofulvin (*d*-2) with EtSH Under an Ar atmosphere, EtSH (2.20 ml, 29.8 mmol) was added dropwise to a solution of *d*-**2**[9] (2.10 g, 5.95 mmol) and *p*-TsOH (1.09 g, 5.95 mmol) in dry CH_2Cl_2 (15 ml). The reaction mixture was stirred at room temperature for 40 min, then aqueous NaOCl solution (50 ml) was added and the whole was acidified with 10% aqueous KOH solution, then extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl solution, dried, and evaporated. The ratio (1 : 2) of *l*-2′-demethoxy-2′-(ethylthio)epigriseofulvin (*l*-**14d**) and *d*-4′-demethoxy-4′-(ethylthio)epi-isogriseofulvin (*d*-**15d**) contained in the residue was determined by HPLC (column, Chemcosorb 5Si-U; column temperature, room temperature; eluent, AcOEt:hexane = 4:1; flow rate, 1.0 ml/min; wavelength, 254 nm). Separation and purification of the residue by column chromatography (eluent: AcOEt : hexane = 1 : 1) gave *d*-**15d** and *l*-**14d** (eluent: AcOEt : hexane = 1 : 1).

l-**14d** (0.321 g, 14%) as colorless needles, mp 182—184 °C (CH_2Cl_2 and Et_2O). $[\alpha]_D^{15}$ −57° (c = 0.010, $CHCl_3$). IR (KBr) *v*: 1715, 1630 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.08 (3H, t, J = 6.8 Hz, C6′-CH_3), 1.26 (3H, t, J = 7.4 Hz, SCH_2CH_3), 2.47 (1H, dd, J = 17.0, 7.7 Hz, C5′-HH), 2.64 (1H, dqd, J = 7.7, 6.8, 4.9 Hz, C6′-H), 2.75, 2.76 (each 1H, each dq, J = 12.6, 7.4 Hz, SCHHCH$_3$), 2.92 (1H, dd, J = 17.0, 4.9 Hz, C5′-HH), 3.98, 4.02 (each 3H, each s, each OCH_3), 5.99 (1H, s, C3′-H), 6.14 (1H, m, C5-H). EI-MS *m/z*: 384 $[M^+ + 2]$, 382 $[M^+]$. *Anal.* Calcd for $C_{18}H_{19}ClO_5S$: C, 56.47; H, 5.00. Found: C, 56.09; H, 5.09.

d-**15d** (0.969 g, 14%) as colorless needles, mp 207—209 °C (CH_2Cl_2 and Et_2O), $[\alpha]_D^{15}$ +67° (c = 0.010, $CHCl_3$). IR (KBr) *v*: 1725, 1640 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.02 (3H, t, J = 6.6 Hz, C6′-CH_3), 1.26 (3H, t, J = 7.4 Hz, SCH_2CH_3), 2.62 (1H, ddd, J = 17.5, 8.9, 1.2 Hz, C5′-HH), 2.74—2.83 (2H, m, C5′-HH, C6′-H), 2.86, 2.88 (each 1H, each dq, J = 12.8, 7.4 Hz, SCHHCH$_3$), 3.94, 3.97 (each 3H, each s, each OCH_3), 5.93 (1H, s, C3′-H), 6.09 (1H, m, C5-H). EI-MS *m/z*: 384 $[M^+ + 2]$, 382 $[M^+]$. *Anal.* Calcd for $C_{18}H_{19}ClO_5S$: C, 56.47; H, 5.00. Found: C, 56.58; H, 4.97.

Determination of the Structures of *d*-14a, 14b and *l*-14d. General Procedure Under an Ar atmosphere, *m*-CPBA (65.2 mg, 0.378 mmol) was added to a solution of **14b** (100 mg, 0.252 mmol) in CH_2Cl_2 (4.5 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, then 10% aqueous Na_2SO_3 was added and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$, and a saturated NaCl solution, dried, and evaporated *in vacuo*. Under an Ar atmosphere, NaOMe (0.30 M in MeOH, 1.0 ml, 0.30 mmol) was added dropwise to a solution of the residue in dry benzene (0.9 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, and poured into ice water. The whole was washed with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated *in vacuo*. Recrystallization of the residue from a mixture of CH_2Cl_2 and Et_2O gave *dl*-**5**.

By the same procedure, *d*-**14a** and *l*-**14d** gave *d*-**1** and *d*-**2**, respectively.

Transformation of *d*-15a, 15b, 15c, and *d*-15d to the Corresponding Isogriseofulvin. General Procedure Under an Ar atmosphere, *m*-CPBA (52.2 mg, 0.302 mmol) was added to a solution of **15b** (80 mg, 0.202 mmol) in CH_2Cl_2 (3.5 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min, then 10% aqueous Na_2SO_3 was added and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ and saturated NaCl solution, dried, and evaporated *in vacuo*. Under an Ar atmosphere, NaOMe solution (0.30 M in MeOH, 0.8 ml, 0.24 mmol) was added dropwise to a solution of the residue in dry benzene (0.8 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and poured into ice water. The whole was washed with 10% aqueous HCl solution and extracted with AcOEt. The AcOEt layer was washed with water, dried, and evaporated in vacuo. Recrystallization of the residue from a mixture of CH_2Cl_2 and petroleum ether gave **16b**.

By the same procedure, *d*-**15a**, **15c** and *d*-**15d** gave *d*-**16a**, **16c** and

d-**16d**, respectively.

d-Isogriseofulvin (*d*-**16a**, 52%) as colorless needles, mp 182—184 °C (lit.[8] 200—201 °C).

dl-6′-Demethyl-6′-ethylisogriseofulvin (**16b**, 66%) as colorless needles, mp 219—223 °C. IR (KBr) *v*: 1700, 1655 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.90 (3H, t, J=7.5 Hz, $CH_2C\underline{H}_3$), 1.18—1.28, 1.54—1.63 (each 1H, each m, $C\underline{H}_2CH_3$), 2.56—2.65 (2H, m, C5′-$\underline{H}$H and C6′-$\underline{H}$), 3.06 (1H, dd, J=18.9, 13.3 Hz, C5′-H$\underline{H}$), 3.77 (3H, s, C3′-$OC\underline{H}_3$), 3.91, 3.99 (each 3H, each s, each $OC\underline{H}_3$), 5.44 (1H, s, C3′-$\underline{H}$), 6.07 (1H, s, C5-$\underline{H}$). EI-MS *m/z*: 368 [M^++2], 366 [M^+]. *Anal.* Calcd for $C_{18}H_{19}ClO_5S$: C, 58.94; H, 5.22. Found: C, 58.35; H, 5.15.

dl-6′-Demethyl-6′-phenylisogriseofulvin (**16c**, 68%) as colorless needles, mp 207—208 °C. IR (Nujol) *v*: 1705, 1660 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.24—4.01 (3H, m, C5′-$\underline{H}\underline{H}$ and C6′-$\underline{H}$), 3.79 (3H, s, C3′-$OC\underline{H}_3$), 3.85 (6H, s, $OC\underline{H}_3\times2$), 5.56 (1H, s, C3′-$\underline{H}$), 5.94 (1H, s, C5-$\underline{H}$). FAB-MS (positive ion mode) *m/z*: 417 [$(M+1)^+$+2], 415 [$(M+1)^+$]. *Anal.* Calcd for $C_{22}H_{19}ClO_6\cdot2H_2O$: C, 58.61; H, 5.14. Found: C,58.92; H, 4.48.

d-Epiisogriseofulvin (*d*-**16d**, 37%) as colorless needles, mp 200—202 °C (lit.[9] 200—202 °C).

Antifungal Activity Assays and evaluation of antifungal activities were carried out according to the methods described previously.[1]

References

1) Tomozane H., Takeuchi Y., Choshi T., Kishida S., Yamato M., *Chem. Pharm. Bull.*, **38**, 925—929 (1990).
2) *a*) Mulholland T. P. C., Honeywood R. I. W., Preston H. D., Rosevear D. T., *J. Chem. Soc.*, **1965**, 4939—4953; *b*) Brossi A., Baumann M., Burkhardt F., *Helv. Chim. Acta*, **45**, 1292—1297 (1962).
3) *a*) Yamato M., Takeuchi Y., Tomozane H., *Synthesis*, **1990**, 569—570; *b*) Takeuchi Y., Watanabe I., Tomozane H., Hashigaki K., Yamato M., *Chem. Pharm. Bull.*, **39**, 3048—3050 (1991).
4) Koe B. K., Celmer W. D., *J. Med. Chem.*, **7**, 705—709 (1964).
5) Mukaiyama T., Kobayashi S., Murakami M., *Chem. Lett.*, **1984**, 1759—1762.
6) Stork G., Tomasz M., *J. Am. Chem. Soc.*, **84**, 310—312 (1962).
7) Brossi A., Baumann M., Gerecke M., Kyburz E., *Helv. Chim. Acta*, **43**, 1444—1447 (1960).
8) Grove J. F., MacMillan J., Mulholland T. P. C., Rogers M. A. T., *J. Chem. Soc.*, **1952**, 3949—3958.
9) MacMillan J., *J. Chem. Soc.*, **1959**, 1823—1830.
10) Weber K., Wehland J., Herzog W., *J. Mol. Biol.*, **102**, 817—829 (1976).