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Microbial Transformation of (+)- and (−)-2′-Demethoxydehydrogriseofulvin by *Streptomyces cinereocrocatus*

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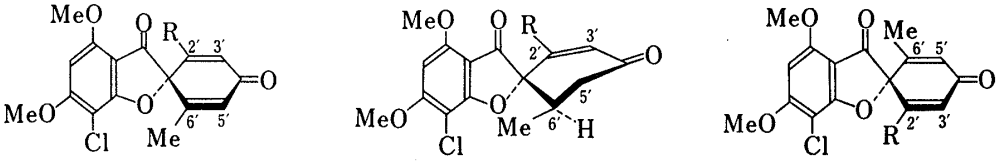
To elucidate the microbial activities of *Streptomyces cinereocrocatus*, (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1c** and **3c**) were synthesized and subjected to microbial transformation. The microbial transformation of the (−)-enantiomer (**3c**) afforded (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18**) as the sole product in a high yield (60%). On the other hand, the microbial transformation of the (+)-enantiomer (**1c**) gave (+)-2′-demethoxygriseofulvin (**6**) (12%) and a mixture of (−)- and (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**14** and **18**) (8%). The results indicate that the microbial transformations take place directly and/or after isomerization with hydrogenations, depending on the kinds of substrates concerned, *i.e.*, (+)- and (−)-2′-demethoxydehydrogriseofulvin analogs. It was concluded that the modes of microbial transformation of dehydrogriseofulvin analogs are greatly influenced by the structure of the substrates, *i.e.* the substituent at the 2′-position and/or the enantiomeric nature.

Keywords——microbial transformation; *Streptomyces cinereocrocatus*; CD; optical rotation; enantiomer; (+)-2′-demethoxygriseofulvin; (−)-2′-demethoxygriseofulvin; (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin; (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin; deuterated derivative; conformation

In a preceding paper[1] we deduced by means of ^2H nuclear magnetic resonance (NMR) spectroscopy that (−)-[5′-^{2}H]dehydrogrisefulvin (DGF) is transformed into (+)-[5′α-^{2}H]-griseofulvin (GF) by *Streptomyces cinereocrocatus*. Moreover, we have proved[2] that the microbial transformation products of (+)-DGF (**3a**) and (+)-[5′-^{2}H]DGF are natural (+)-GF and (+)-[5′α-^{2}H]GF, respectively, demonstrating in consequence that (−)- and (+)-[5′-^{2}H]DGF are both transformed into (+)-[5′α-^{2}H]GF by *Streptomyces cinereocrocatus*. The results of incubations of (−)- and (+)-DGF with eight *Streptomyces* species have shown[2] that (−)- and (+)-DGF are interconvertible and are both converted to (+)-GF as the main product. Further, we have proved[3] that the 2′-propoxy analogs (**1b** and **3b**) of (−)- and (+)-DGF were transformed into the same product, the 2′-propoxy analog of (+)-GF by *S. cinereocrocatus* (Chart 1).



1a: R=OMe
1b: R=OPr
1c: R=H

2a: R=OMe
2b: R=OPr
2c: R=H

3a: R=OMe
3b: R=OPr
3c: R=H

Chart 1

In this communication, we describe studies which demonstrate that the microbial transformations of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1c** and **3c**) by *Streptomyces cinereocrocatus* take place directly and/or after isomerization with hydrogenations, providing further examples which indicate that the mode of the microbial transformation is greatly influenced by minor changes in the structure of substrates.

Results and Discussion

Syntheses of Substrates for Microbial Transformation

Substrates (**1c** and **3c**) were prepared from (+)-GF (**2a**) (Chart 2). In order to determine the configuration of the 2′-methoxy group of (−)-dihydrogriseofulvin (**4**),[4a] which is the reduction product of **2a** and also an important intermediate for the preparation of (+)-2′-demethoxydehydrogriseofulvin (**1c**), **4** was treated with selenium dioxide in *tert*-butanol to afford (−)-2′,3′-dihydrodehydrogriseofulvin (**5**) along with **2a**. The ^{1}H-NMR of **5** exhibited a three-proton singlet signal (1.82 ppm) due to the 6′-methyl protons and an olefinic proton signal (6.09 ppm) due to the 5′-proton, and the signal of the C-2′ proton showed a coupling pattern (J=12.5 and 6.0 Hz) suggesting it to have α and axial configuration. Further, comparison of the ^{1}H-NMR and circular dichroism (CD) data of **5** with those of **21**, described below (Chart 3), indicated that the 2′-methoxy group of **5** has β-configuration and is equatorial. Thus, the stereochemistry of **4** was established to be as shown in Chart 2 by considering its ^{1}H-NMR signals in comparison with those of **5**. (+)-2′-Demethoxygriseofulvin (**6**) was prepared by acid treatment of **4** according to the method of Mulholland.[4b] In the ^{1}H-NMR spectrum of **6**, the 2′-methoxy signal (3.27 ppm) of **4** had disappeared, and there were two new doublet signals (6.55 and 6.19 ppm) which were assignable to 2′-H and 3′-H. The optical rotation and the other physical data indicated that **6** is (+)-2′-demethoxygriseofulvin. Treatment of **6** with pyridinium hydrobromide perbromide ($PyHBr_3$)[5] in chloroform furnished a mixture of (+)-3′-bromo-2′-demethoxygriseofulvin (**7**) and (+)-5′α-bromo-2′-demethoxygriseofulvin in a ratio of 40: 60. Their structures were determined by ^{1}H-NMR analyses: the ^{1}H-NMR of **7** exhibited only one proton signal in an olefinic region and that of **8** showed a doublet signal at 5.27 ppm (J=13 Hz) assignable to 5′β-H. Accordingly, (+)-2′-demethoxydehydrogriseofulvin (**1c**), which is one of the desired substrates, was prepared by dehydrobromination[6] of the 5′α-bromo derivative (**8**) with LiCl and Li_2CO_3 in *N*-dimethyl formamide (DMF) containing

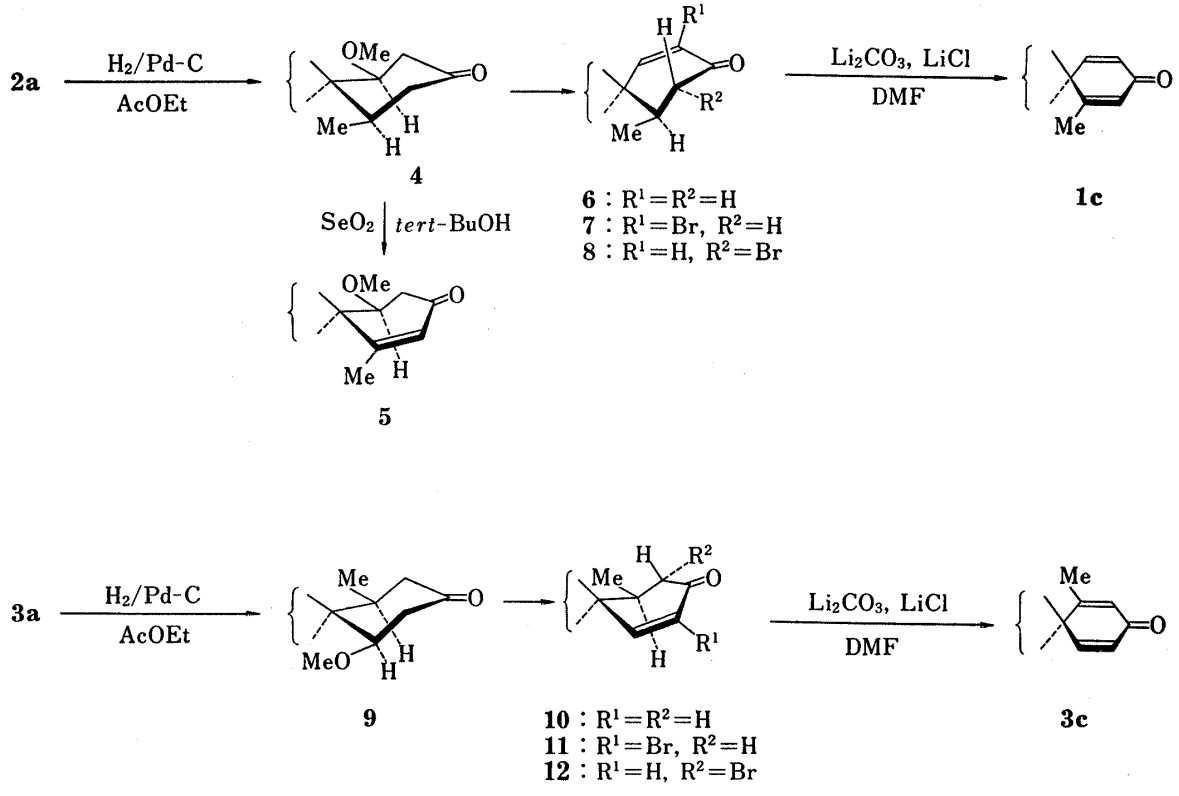


Chart 2

pyridine, and the structure was confirmed by the mass spectrum (MS) (M^+; m/z 320), CD spectrum and ^{1}H-NMR spectrum, in which the signals due to 5′β-H and 6′α-H and a doublet due to 6′-methyl of **8** had disappeared and a signal (6.42 ppm) assignable to olefinic 5′-H and a doublet signal at 1.82 ppm (J=2.0 Hz) due to 6′-methyl on the olefinic carbon were seen.

Next, (−)-2′-demethoxydehydrogriseofulvin (**3c**), the enantiomer of **1c** was synthesized from (+)-GF (**2a**) as follows (Chart 2). (+)-DGF (**3a**) was prepared as the starting material from (+)-GF as described previously.[2] Hydrogenation of **3a** with palladium-charcoal catalyst in ethyl acetate resulted in the formation of a mixture of two products (55: 45, **9** and griseophenone A[2]), from which the desired neutral product (**9**) was isolated. The ^{1}H-NMR and MS data of **9** were identical with those of (−)-dihydrogriseofulvin (**4**). However, the CD and optical rotation together with the above data clearly indicated that **9** was (+)-dihydrogriseofulvin, the enantiomer of **4**. Consequently, (−)-2′-demethoxydehydrogriseofulvin (**3c**) was finally synthesized from **9** as described above; that is, (−)-2′-demethoxygriseofulvin (**10**) was prepared by the acid treatment of **9**, and a solution of **10** and $PyHBr_3$ in chloroform was reacted under reflux to give a mixture of (−)-3′-bromo-2′-demethoxygriseofulvin (**11**) and (−)-5′α-bromo-2′-demethoxygriseofulvin (**12**), which can be separated by fractional crystallization and silica gel column chromatography. Subsequent dehydrobromination of the 5′α-bromo derivative (**12**) with lithium salts in DMF containing pyridine yielded (−)-2′-demethoxydehydrogriseofulvin (**3c**). The spectral and physical data of the compounds (**10**, **12** and **3c**) were identical with those of the corresponding enantiomers (**6**, **8** and **1c**), except for the CD and optical rotation data (Figs. 1 and 2b).

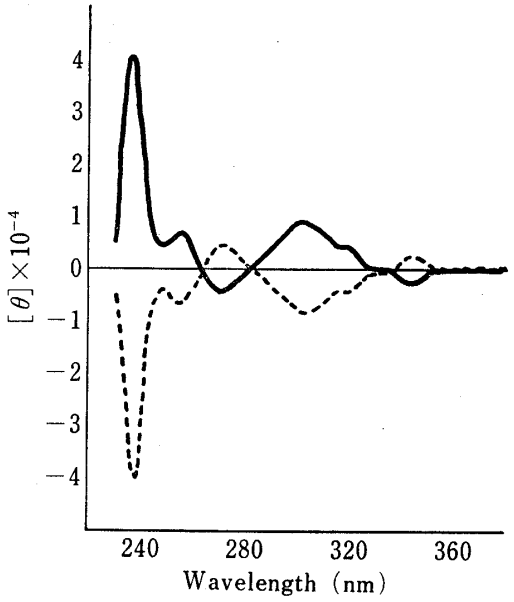


Fig. 1. CD Spectra of Substrates **1c** (——) and **3c** (……)

Syntheses of Standard Compounds

The six compounds (**6**, **10**, **14**, **16**, **17**, and **18**) which are possible microbial transformation products were synthesized as follows. Two ,**6** and **10**, were prepared as described above (Chart 2). The syntheses of the other compounds are shown in Chart 3. (+)-2′-Demethoxydihydrogriseofulvin (**13**) was prepared by catalytic hydrogenation over palladium-charcoal of **6** in ethyl acetate according to the method of Dawkins *et al.*[7] Then, a solution of **13** and selenium dioxide in *tert*-butanol was refluxed to give a mixture of **14** and **6** (36: 64), which was separated by silica gel column chromatography. In the ^{1}H-NMR spectrum of **14**, there appeared a three-proton doublet signal at 1.79 ppm (J=1.8 Hz) assignable to the 6′-methyl protons and a broad singlet signal at 6.10 ppm assignable to the 5′-olefinic proton. The ^{1}H-NMR and other physical data demonstrated that **14** is (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin. Further, the catalytic hydrogenation of **14** gave a mixture of **13** and **15** (53: 47), which was separated by silica gel column chromatography. By examination of the ^{1}H-NMR and MS data, **15** was identified as (+)-2′-demethoxy-2′,3′-dihydroepigriseofulvin, the epimer of **13**. Dehydrogenation of **15** with selenium dioxide in *tert*-butanol gave a mixture of **16** and **14** (50: 50), which was subjected to silica gel column chromatographic separation. The ^{1}H-NMR spectrum of **16** indicated that it is (+)-2′-demethoxyepigriseofulvin.

On the other hand, **17** and **18** were synthesized as follows. First, dihydroepigriseofulvin (**19**) was prepared by catalytic hydrogenation of (+)-epigriseofulvin (**20**)[2] in ethyl acetate.

In order to clarify the configuration of the 2′-methoxy group of **19**, **21** was prepared by dehydrogenation of **19** with selenium dioxide in *tert*-butanol. Comparison of its ^{1}H-NMR with that of **5** clearly indicated that the configuration of the 2′-methoxy group of **21** is α. Consequently,

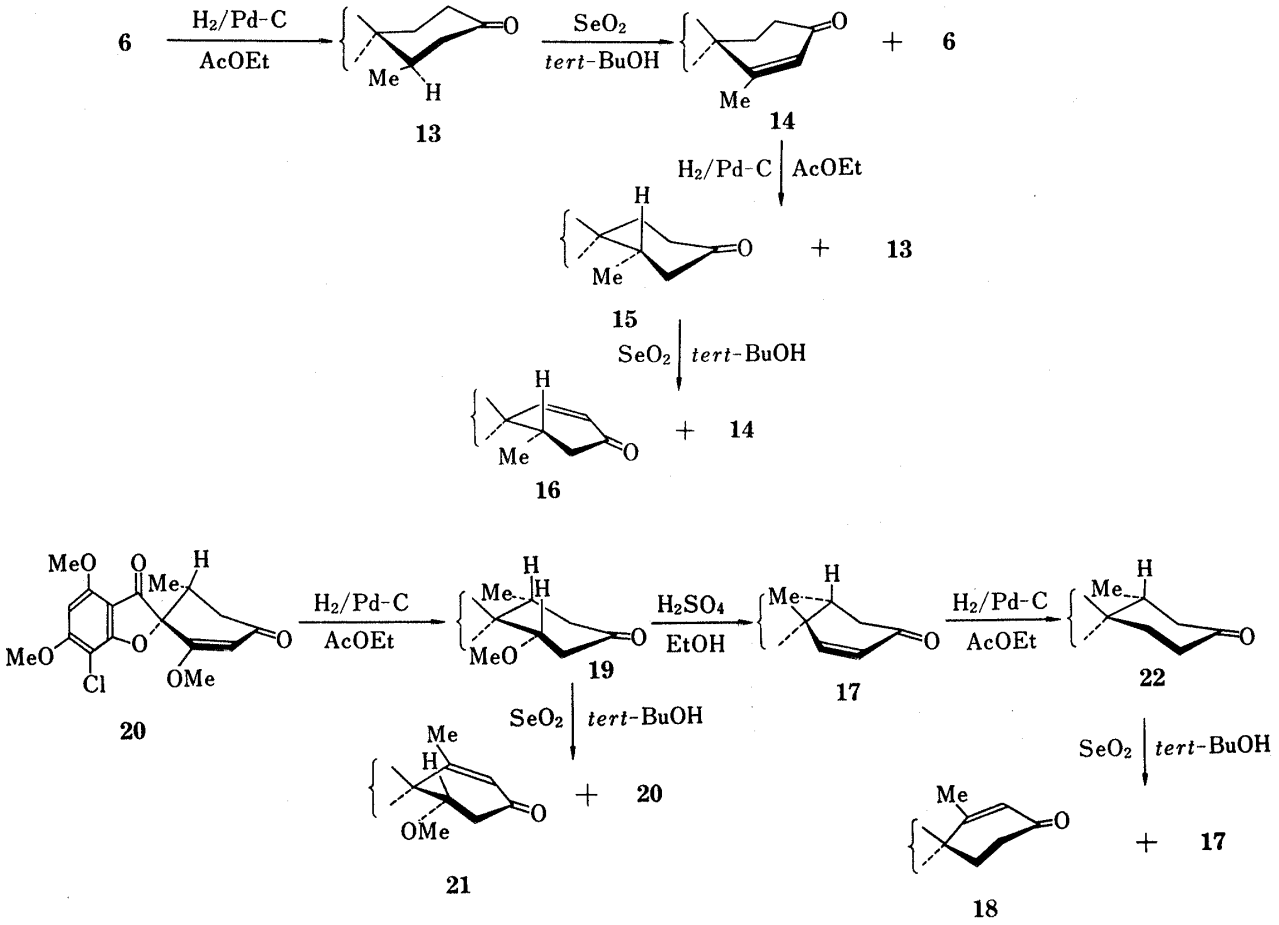


Chart 3

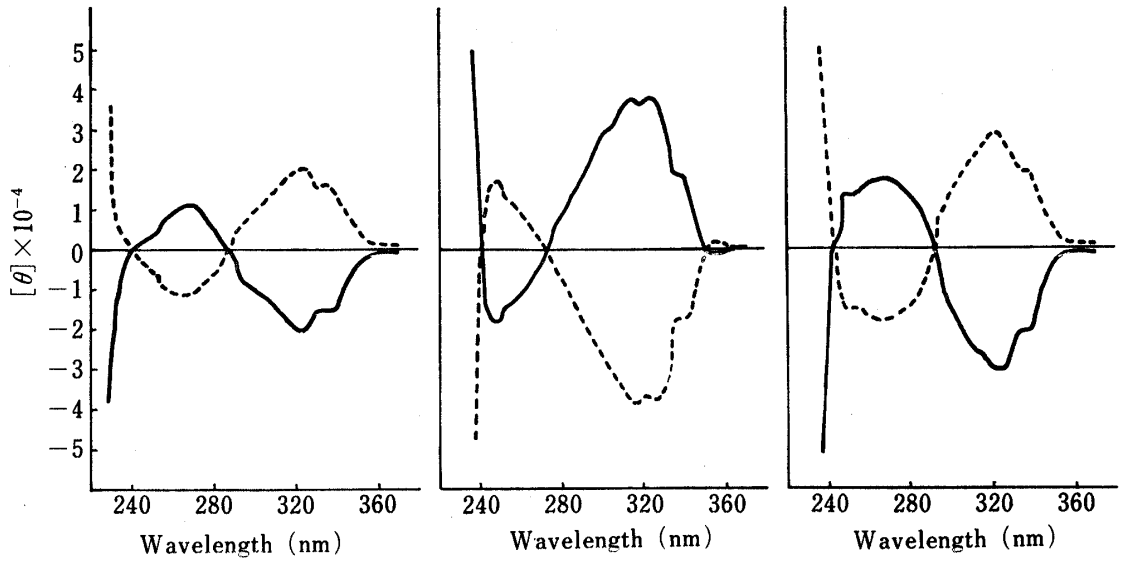


Fig. 2a. CD Spectra of Compounds **17** (——) and **16** (·····) in $CHCl_3$

Fig. 2b. CD Spectra of Compounds **6** (——) and **10** (·····) in $CHCl_3$

Fig. 2c. CD Spectra of Compounds **14** (——) and **18** (·····) in $CHCl_3$

19 was unequivocally identified as dihydroepigriseofulvin with a 2′α-methoxy group. Next, (−)-2′-demethoxyepigriseofulvin (**17**) was prepared by acid treatment of **19**. The ^{1}H-NMR and mass spectral data were identical with those of **16**. However, the CD (Fig. 2a) and optical rotation together with the above data clearly indicated that **17** was (−)-2′-demethoxyepigriseofulvin, the enantiomer of **16**. The catalytic hydrogenation of **17** furnished (−)-2′-demethoxydihydroepigriseofulvin (**22**), the enantiomer of **15**. Dehydrogenation of **22** with selenium dioxide in *tert*-butanol gave a mixture of **18** and **17** (50: 50), which was subjected to silica gel column chromatographic separation. Comparison of the physical data with those of **14** clearly demonstrated that **18** was (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin, the enantiomer of **14** (Fig. 2c).

The Conformation of (−)-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (14)

Since the microbial hydrogenations of **1c** and **3c** yielded new products reduced at the 2′,3′-unsaturated bond, it was necessary to determine the conformation of 2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**14**) in order to elucidate the stereochemistry of the microbial

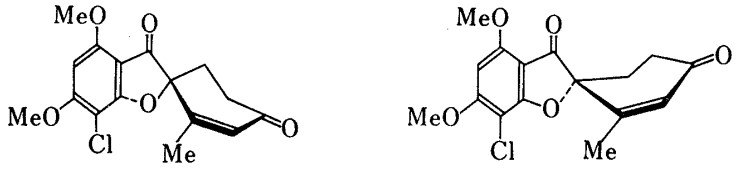


Chart 4

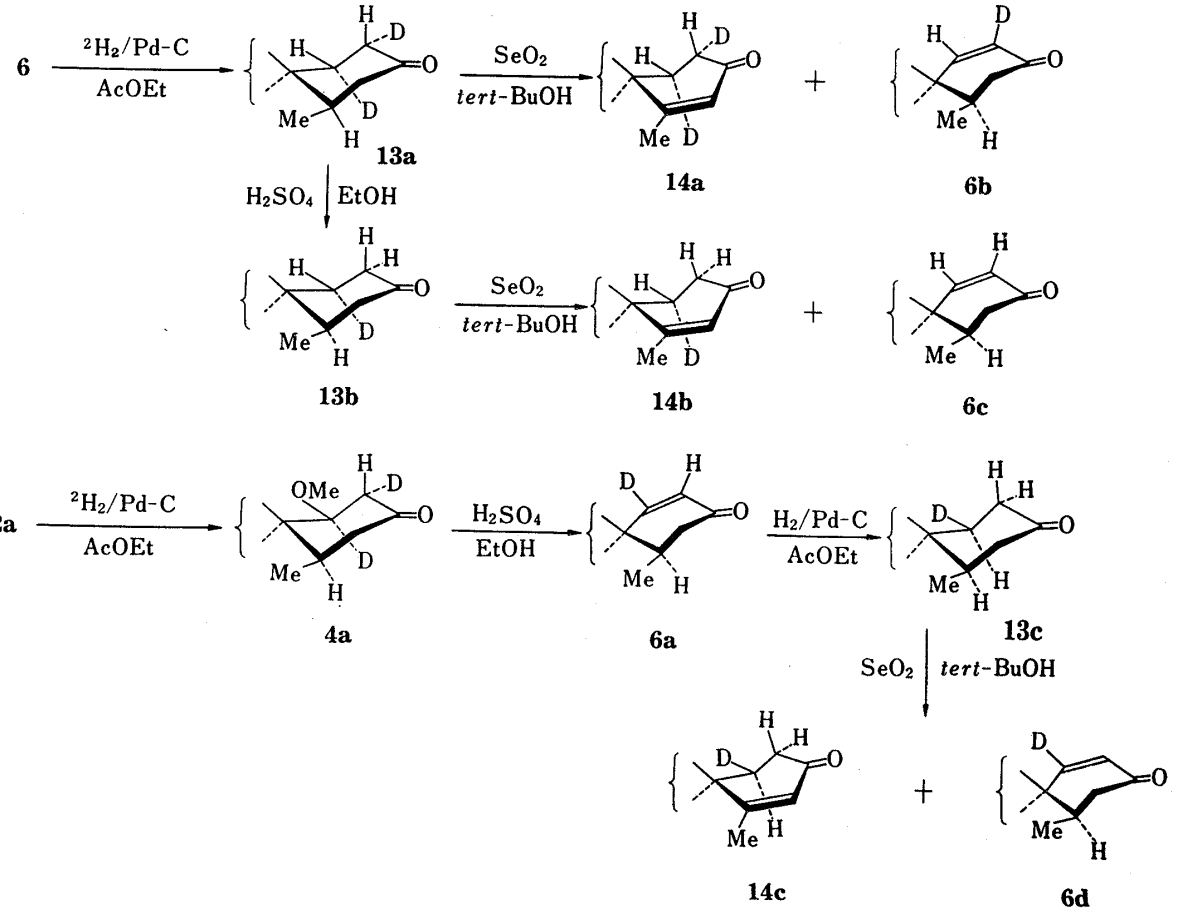


Chart 5

hydrogenation of **1c** and **3c**. Previously,[3] we suggested that the conformation of **14** is of A type shown in Chart 4 based on the CD data of 2′-demethoxygriseofulvin, 2′-demethoxyepigriseofulvin and related compounds. In order to judge the conformation on the basis of ^{1}H-NMR data, deuterated derivatives at the 2′- and/or 3′-position(s) of **13** (**13a**—**c**) and of **14** (**14a**—**c**) were synthesized as shown in Chart 5. Fig. 3 shows parts of the ^{1}H-NMR spectra of some of the deuterated compounds (**13a**—**c**) and **13**. Inspection of the ^{1}H-NMR signals shown in Fig. 3b and 3c, together with those of protons on C-3′ revealed the patterns of a doublet ($J_{2'\beta-3'\beta}$=6.0 Hz) of **13a** and a broad triplet of 2′β-H as a result of a double-doublet ($J_{2'\beta-3'\beta}=J_{2'\beta-3'\alpha}$=6.0 Hz) of **13b**. These results clearly indicate that **13a** and **13b** are [2′α,3′α-$^2H_2$]- and [2′α-^{2}H]-**13**, respectively. On the other hand, the ^{1}H-NMR spectrum (Fig. 3d) of **13c** obtained by catalytic hydrogenation of [2′-^{2}H]-2′-demethoxygriseofulvin (**6a**) over palladium-charcoal exhibited a double-doublet ($J_{2'\alpha-3'\beta}$=8.0 Hz and $J_{2'\alpha-3'\alpha}$=5.4 Hz) which demonstrates the structure of **13c** to be [2′β-^{2}H]-**13**.

Fig. 4 shows parts (2′β, 2′α, 3′α, and 3′β proton regions) of the ^{1}H-NMR spectra of several of the deuterated compounds (**14a**—**c**) and **14**. Dehydrogenation of the deuterated compounds (**13a**—**c**) with selenium dioxide afforded the corresponding unsaturated compounds (**14a**—**c**), respectively, whose ^{1}H-NMR spectra confirmed the orientations of the deuterium atom(s) in each compound as shown in Chart 5. Inspection of the pattern of each signal in Figs. 3 and 4 and a consideration of the preparation methods indicated that conformation of **14** should be B type instead of A as shown in Chart 4. At the same time, the ^{1}H-NMR data indicated that each catalytic hydrogenation proceeded predominantly from the α-side at the 2′ and 3′ positions. Inspection of the ^{1}H-NMR spectra of **6b**—**d**, which are dehydrogenation products of **13a**—**c**, respectively, proved that they consist mainly of (+)-[3′-^{2}H]-**6**, (+)-undeuterated **6** and (+)-[2′-^{2}H]-**6**, respectively. Further, it was demonstrated that selenium dioxide treatment of the cyclohexanone derivatives (**13a**—**c**) affords the corresponding unsaturated compounds predominantly *via* dehydrogenation in a *trans-diaxial* manner.

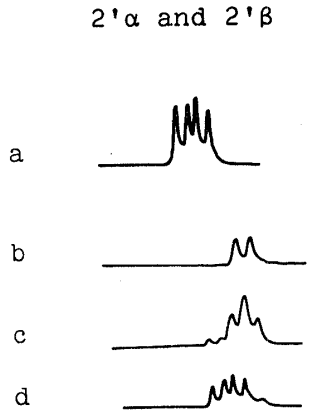


Fig. 3. Parts (2′α and 2′β Proton Region) of the 400 MHz ^{1}H-NMR Spectra of Partially Deuterated Compounds (**13a**, **13b**, and **13c**) and **13**

a, **13**; b, **13a**; c, **13b**; d, **13c**.

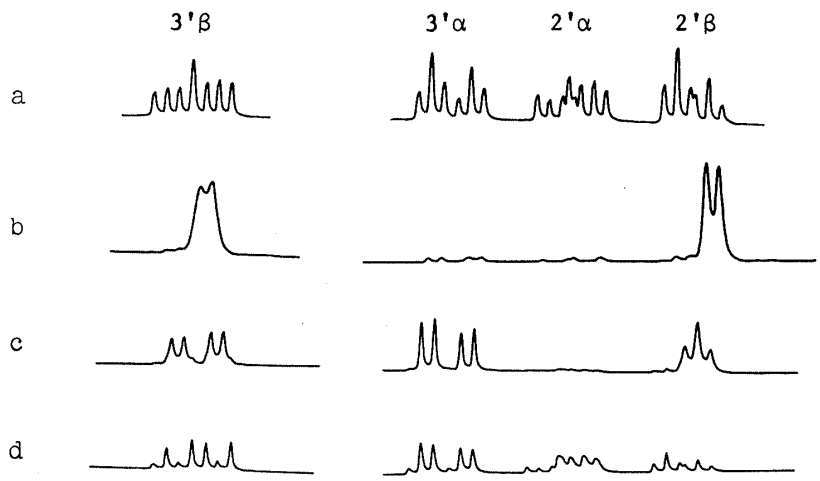


Fig. 4. Parts (2′α, 2′β, 3′α, and 3′β Proton Region) of the 400 MHz ^{1}H-NMR Spectra of Partially Deuterated Compounds (**14a**, **14b** and **14c**) and **14**

a, **14**; b, **14a**; c, **14b**; d, **14c**.

Microbial Transformation of (+)- and (−)-2′-Demethoxydehydrogriseofulvin by *Streptomyces cinereocrocatus*

The microbial transformation of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1c** and **3c**) by *Streptomyces cinereocrocatus* was performed under the same conditions as described in the

previous paper.[2] The yields of the reduction products and the recovered material obtained after the microbial transformations are shown in Fig. 5. The microbial transformation products of **1c** were 2′-demethoxygriseofulvin and 2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin. After 12 h, no substrate was left; the microbial reduction afforded 2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin preferentially over 2′-demethoxygriseofulvin in the 3 and 6 h incubation experiments. The structures of the 12 h incubation products were inferred from the ^{1}H-NMR and mass spectral data to be (+)-2′-demethoxygriseofulvin (**6**) and a mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18** and **14**), whose relative ratio was calculated to be 81 : 19 by comparisons of the CD spectra with those of the standard (+)- and (−)-compounds. On the other hand, the recovered **1c** obtained after 3 h of incubation was optically pure (+)-2′-demethoxydehydrogriseofulvin without any contamination by the enantiomeric (−)-2′-demethoxydehydrogriseofulvin. Moreover, the formation of (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18**) suggests that the microorganism has the abilities to isomerize the substrate (**1c**) into the enantiomer (**3c**) and then to reduce the

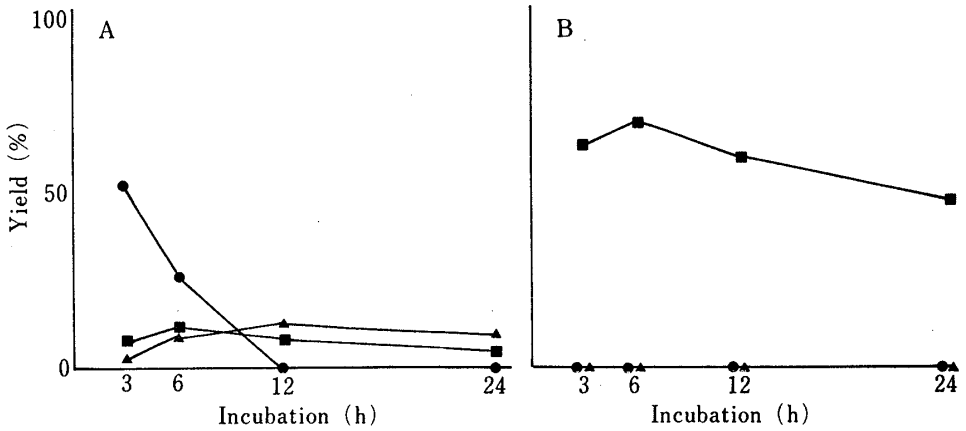


Fig. 5. Time Courses of the Yields of Microbial Transformation Products and Recovered Substrate

A: (+)-2′-Demethoxydehydrogriseofulvin (**1c**) was used as the substrate.
B: (−)-2′-Demethoxydehydrogriseofulvin (**3c**) was used as the substrate.
2′-Demethoxygriseofulvin(—▲—); 2′-demethoxy-2′ 3′-dihydrodehydrogriseofulvin (—■—); 2′-demethoxydehydrogriseofulvin (—●—).

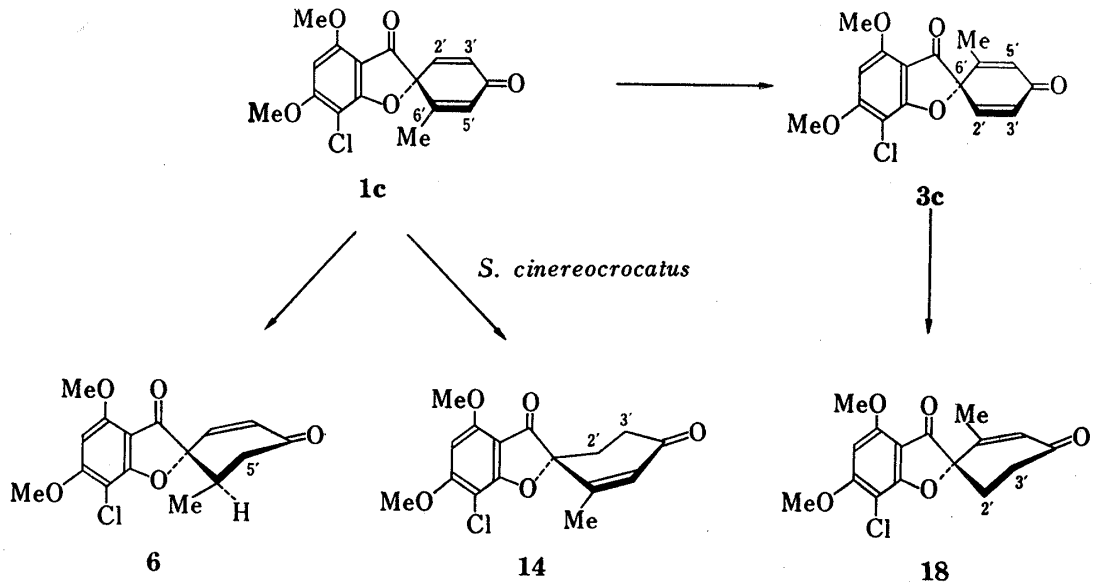


Chart 6

latter. Next, (−)-2′-demethoxydehydrogriseofulvin (**3c**) was subjected to microbial transformation by *S. cinereocrocatus*. The product, 2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin, was obtained in a high yield without recovery of the substrate even after 3 h. The structure was inferred from the ^{1}H-NMR and mass spectral data, and the product was confirmed to be optically pure (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18**) by comparisons of its CD spectrum with those of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18** and **14**). These results are summarized in Chart 6, which indicates that the microbial hydrogenations of **1c** and **3c** yielded a new product reduced at the 2′,3′-unsaturated bond, when compared with our previous results on the microbial transformation.

A comparison of the microbial transformations of (−)- and (+)-DGF with those of the 2′-propoxy analogs of (−)- and (+)-DGF by *S. cinereocrocatus* showed that the microbial transformation modes of the latter are the same as those of the former, although the rate of microbial transformations of the 2′-propoxy analogs was much lower.[3] Hence, we conclude that in microbial treatment with *S. cinereocrocatus*, the analogs of (−)- and (+)-DGF which have or do not have a substituent at the 2′-position are hydrogenated directly or after isomerization into the corresponding enantiomers, yielding (+)- and/or (−)-dihydro compound(s) as the transformation products. These results indicate that the microbial transformation of DGF analogs is affected by both the substituent at the 2′-position of the DGF skeleton and the enantiomeric structure.

Experimental

All melting points were obtained on a micro-melting point apparatus, type MM2 (Shimadzu Seisakusho Ltd.), and are uncorrected. Analytical gas chromatography was carried out on a Shimadzu GC-6A gas liquid chromatography by using a flame ionization detector and the carrier gas was nitrogen. A glass column (2 m×3 mm i.d.) of 1.5% OV-17 on Chromosorb W was used. ^{1}H-NMR spectra were obtained on JEOL JNM-MH-100 and JEOL FX-400 NMR machines. All ^{1}H-NMR spectra were recorded in deuteriochloroform and reported as parts per million downfield from Me_4Si (δ=0). Abbreviations used: s=singlet, d=doublet, t=triplet, br=broad, m=multiplet, and dd=double doublet. MS were recorded on a JEOL D-100 spectrometer at 75 eV ionizing potential and are reported as *m/z*. Optical rotations were measured on a JASCO DIP-SL automatic polarimeter with a cell of 10-cm light path length, and CD spectra were taken in a 0.5-mm cell at room temperature (24—25°C) in chloroform on a JASCO J-20 recording spectropolarimeter. CD spectra were recorded with 4 accumulations from 380 to 230 nm. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh). Thin layer chromatography was carried out by using 5×20 cm plates, 0.25-mm thickness, of Merck silica gel 60F-254. Compounds were analyzed by developing the plates with benzene–acetone (7: 3 v/v), and spots were visualized under ultraviolet light and/or by heating on an electric heater after spraying with conc. H_2SO_4.

(−)-Dihydrogriseofulvin (4)——**4** was prepared essentially by the method of Mulholland.[4a] Recrystallization from benzene gave colorless needles, mp 210—211°C (lit.[4a] 198°C), $[\alpha]_D^{18}$ −24.5° (*c*=0.60, acetone). *Anal.* Calcd for $C_{17}H_{19}ClO_6$: C, 57.55; H, 5.40. Found: C, 57.98; H, 5.42. MS *m/z*: 354 (M^+) (for ^{35}Cl-compound), 322, 255 (base peak). ^{1}H-NMR δ (ppm): 0.92 (3H, d, *J*=6.4 Hz, 6′β-Me), 2.31 (1H, ddd, *J*=14.0, 6.0 and 2.5 Hz, 5′α-H), 2.36—2.44 (1H, m, 6′α-H), 2.79 (1H, ddd, *J*=14.0, 6.0 and 2.5 Hz, 3′α-H), 3.15 (1H, dd, *J*=14.0 and 14.0 Hz, 5′β-H), 3.31 (3H, s, 2′β-OMe), 3.32 (1H, dd, *J*=14.0 and 12.5 Hz, 3′β-H), 3.80 (1H, dd, *J*=12.5 and 6.0 Hz, 2′α-H), 4.00 (3H, s, 4-OMe), 4.40 (3H, s, 6-OMe), 6.14 (1H, s, 5-H). Molecular ellipticity [θ] (*c*=1.0 mg/ml): $[\theta]_{360}$ 0, $[\theta]_{339}$ −8780, $[\theta]_{334}$ −7680, $[\theta]_{326}$ −9310, $[\theta]_{303}$ 0, $[\theta]_{291}$ +7970, $[\theta]_{255}$ 0, $[\theta]_{240}$ −15290, $[\theta]_{233}$ 0, $[\theta]_{230}$ +11330.

(−)-2′,3′-Dihydrodehydrogriseofulvin (5)——A solution of **4** (500 mg) and selenium dioxide (500 mg) in *tert*-butanol (20 ml) was refluxed for 10 h. The ratio (85: 15, **5** and **2a**) of the products in the reaction mixture was determined by gas liquid chromatography. The hot reaction mixture was filtered and Darco-G-60 (500 mg) was added. The mixture was filtered again after 20 min. After removal of the solvent under reduced pressure and extraction of the residue with benzene, the benzene extract was directly subjected to column chromatography over silica gel (50 g) in benzene. 1) Elution with benzene–methylene chloride (50: 50), gave an eluate which yielded colorless needles of (−)-2′,3′-dihydrodehydrogriseofulvin (**5**). This product was recrystallized from benzene, mp 210—211°C, $[\alpha]_D^{24}$ −409.3° (*c*=0.14, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 58.19; H, 4.84. MS *m/z*: 352 (M^+) (for ^{35}Cl-compound), 317, 294 (base peak), 266, 251, 138. ^{1}H-NMR δ (ppm): 1.82 (3H, s, 6′-Me), 2.80 (1H, dd, *J*=16.5 and 6.0 Hz, 3′α-H), 3.25 (1H, dd, *J*=16.5 and 12.5 Hz, 3′β-H), 3.34 (3H, s, 2′-OMe), 3.99 (3H, s, 4-OMe), 4.06 (3H, s, 6-OMe), 4.19 (1H, dd, *J*=12.5 and 6.0 Hz, 2′α-H), 6.09 (1H, br s, 5′-H), 6.16 (1H, s, 5-H). Molecular ellipticity [θ]

(c=1.0 mg/ml): $[\theta]_{375}$ 0, $[\theta]_{354}$ +1690, $[\theta]_{349}$ 0, $[\theta]_{335}$ −23580, $[\theta]_{320}$ −40660, $[\theta]_{314}$ −37950, $[\theta]_{310}$ −39140, $[\theta]_{276}$ 0, $[\theta]_{259}$ +16720, $[\theta]_{246}$ 0. 2) Elution with benzene–methylene chloride (40: 60) gave (+)-griseofulvin (**2a**).

(+)-2′-Demethoxygriseofulvin (6)——**6** was prepared essentially by the method of Mulholland[4b] from **4**. Recrystallization from methanol gave colorless needles, mp 183—184°C (lit.,[7b] 177—178°C), $[\alpha]_D^{18}$ +412.0° (c=0.20, acetone or $CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 59.00; H, 4.52. MS m/z: 322 (M^+) (for ^{35}Cl-compound), 214 (base peak). ^{1}H-NMR δ (ppm): 0.93 (3H, d, J=6.2 Hz, 6′β-Me), 2.45 (1H, dd, J=16.2 and 4.2 Hz, 5′α-H), 2.88 (1H, m, 6′α-H), 3.12 (1H, dd, J=16.2 and 14.0 Hz, 5′β-H), 3.98 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.15 (1H, s, 5-H), 6.19 (1H, d, J=10.0 Hz, 3′-H), 6.57 (1H, d, J=10.0 Hz, 2′H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{376}$ 0, $[\theta]_{354}$ −2090, $[\theta]_{349}$ 0, $[\theta]_{337}$ +17390, $[\theta]_{336}$ +17230, $[\theta]_{322}$ +37670, $[\theta]_{316}$ +35420, $[\theta]_{312}$ +36710, $[\theta]_{274}$ 0, $[\theta]_{247}$ −18290, $[\theta]_{241}$ 0, $[\theta]_{230}$ +128800.

(+)-5′α-Bromo-2′-demethoxygriseofulvin (8)——A mixture of **6** (600 mg) and $PyHBr_3$ (720 mg)[5] in chloroform (120 ml) was refluxed for 2 h. The reaction mixture was poured into a large volume of ice and water, and extracted with chloroform (200 ml×3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 871 mg). The ratio (40: 60) of the two products was determined by gas liquid chromatography. The mixture was separated by repeated recrystallization from methanol and silica gel column chromatography. The recrystallization from methanol gave (+)-5′α-bromo-2′-demethoxygriseofulvin (**8**) as colorless needles, mp 243—245°C, $[\alpha]_D^{24}$ +439.0° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{14}BrClO_5$: C, 47.84; H, 3.51. Found: C, 47.47; H, 3.39. MS m/z: 400 (M^+) (for ^{35}Cl, ^{79}Br-compound), 321, 280, 214 (base peak). ^{1}H-NMR δ (ppm): 1.13 (3H, d, J=6 Hz, 6′β-Me), 3.03 (1H, m, 6′α-H), 3.97 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 5.27 (1H, d, J=13 Hz, 5′β-H), 6.16 (1H, s, 5-H), 6.29 (1H, d, J=10 Hz, 3′-H), 6.63 (1H, d, J=10 Hz, 2′-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{352}$ +400, $[\theta]_{335}$ +27200, $[\theta]_{321}$ +50000, $[\theta]_{310}$ +46400, $[\theta]_{277}$ 0, $[\theta]_{267}$ −7600, $[\theta]_{259}$ 0, $[\theta]_{253}$ +4400, $[\theta]_{247}$ +2600, $[\theta]_{232}$ +152800, $[\theta]_{230}$ +72800.

The mother liquor was rechromatographed over silica gel and eluted with benzene–methylene chloride (60: 40). The eluate gave colorless needles of (+)-3′-bromo-2′-demethoxygriseofulvin (**7**) which were recrystallized from methanol, mp 211—212°C, $[\alpha]_D^{16}$ +329.7° (c=0.16, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{14}BrClO_5$: C, 47.84; H, 3.51. Found: C, 47.39; H, 3.48. MS m/z: 400 (M^+) (for ^{35}Cl, ^{79}Br-compound), 385, 321 (base peak), 215, 214, 140, 69. ^{1}H-NMR δ (ppm): 0.92 (3H, d, J=6 Hz, 6′β-Me), 2.50—3.30 (3H, m, 5′α-, 5′β- and 6′α-H), 3.97 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.13 (1H, s, 5-H), 7.01 (1H, s, 2′-H).

(+)-2′-Demethoxydehydrogriseofulvin (1c)——A solution of **16** (540 mg), LiCl (58 mg), Li_2CO_3 (100 mg) and pyridine (9 ml) in DMF (40 ml) was heated at 100°C for 24 h. Then, the reaction mixture was poured into ice and water, and extracted with chloroform (80 ml×2). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 346 mg). The residue was chromatographed on silica gel (40 g) and eluted with benzene–methylene chloride (40: 60). The eluate gave colorless needles of (+)-2′-demethoxydehydrogriseofulvin (**1c**) which were recrystallized from benzene, mp 251—252°C, $[\alpha]_D^{16}$ +29.8° (c=0.21, acetone). *Anal.* Calcd for $C_{16}H_{13}ClO_5$: C, 59.92; H, 4.09. Found: C, 59.59; H, 4.05. MS m/z: 320 (M^+) (for ^{35}Cl-compound, base peak), 291, 281, 274, 197, 140. ^{1}H-NMR δ (ppm): 1.82 (3H, d, J=2.0 Hz, 6′-Me), 3.98 (3H, s, 4-OMe), 4.05 (3H, s, 6-OMe), 6.18 (1H, s, 5-H), 6.29 (1H, br s, 5′-H), 6.41 (1H, d, J=10.0 Hz, 3′-H), 6.55 (1H, d, J=10.0 Hz, 2′-H). Molecular ellipticity $[\theta]$ (c=1.1 mg/ml): $[\theta]_{370}$ −290, $[\theta]_{343}$ −3360, $[\theta]_{327}$ 0, $[\theta]_{300}$ +8930, $[\theta]_{284}$ 0, $[\theta]_{270}$ −5440, $[\theta]_{263}$ 0, $[\theta]_{255}$ +6190, $[\theta]_{248}$ +3500, $[\theta]_{238}$ +40030.

(+)-Dihydrogriseofulvin (9)——A suspension of 5% palladium-charcoal catalyst (300 mg) in an ethyl acetate solution (100 ml) of **3a** (500 mg)[2] was shaken under a stream of hydrogen at atmospheric pressure and at room temperature. The hydrogenation was stopped after 5 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue (419 mg) was dissolved in chloroform and washed with 2 N aqueous sodium hydroxide. The neutral material was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 210 mg, 54%); only one peak was found by gas liquid chromatography. The neutral product in benzene was subjected to column chromatography on silica gel (50 g). Elution with benzene–methylene chloride (60: 40) (yield, 124 mg) and recrystallization of the product from benzene gave (+)-dihydrogriseofulvin (**9**) as colorless needles, mp 216—217°C, $[\alpha]_D^{26}$ +29.4° (c=0.50, acetone). *Anal.* Calcd for $C_{17}H_{19}ClO_6$: C, 57.55; H, 5.40. Found: C, 57.98; H, 5.42. ^{1}H-NMR and MS were identical with those of **9**. Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{360}$ 0, $[\theta]_{339}$ +8750, $[\theta]_{334}$ +7630, $[\theta]_{326}$ +9330, $[\theta]_{303}$ 0, $[\theta]_{291}$ −7970, $[\theta]_{255}$ 0, $[\theta]_{240}$ +15260, $[\theta]_{233}$ 0, $[\theta]_{230}$ −12710. The yellow alkaline extract, after acidification with conc. hydrochloric acid, was extracted with methylene chloride. The extract was washed with water, dried and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel to give a pale yellow oil. Crystallization of the oil from benzene afforded griseophenone A as pale yellow needles, mp 217—218°C, undepressed on admixture with an authentic sample.[2]

(−)-2′-Demethoxygriseofulvin (10)——**10** was obtained from **9** under the same conditions as described for the preparation of **6** from **4**. **9** (634 mg) was heated under reflux with 2 N sulfuric acid (77 ml) and ethanol (58 ml) for 6 h. After neutralization with 5 N Na_2CO_3, the solid (623 mg) obtained by concentration *in vacuo* and filtration was chromatographed in benzene on silica gel (50 g) and eluted with benzene–methylene chloride (50: 50). Recrystallization of the product from methanol gave (−)-2′-demethoxygriseofulvin as colorless needles, mp 180—182°C, $[\alpha]_D^{26}$ −410.4° (c=0.11, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 59.54; H, 4.78. ^{1}H-NMR and MS were identical with those of **6**. Molecular ellipticity $[\theta]$ (c=

1.0 mg/ml): $[\theta]_{376}$ 0, $[\theta]_{354}$ +2080, $[\theta]_{349}$ 0, $[\theta]_{337}$ −17370, $[\theta]_{336}$ −17100, $[\theta]_{322}$ −37450, $[\theta]_{316}$ −35450, $[\theta]_{312}$ −35540, $[\theta]_{274}$ 0, $[\theta]_{247}$ +18070, $[\theta]_{241}$ 0, $[\theta]_{230}$ −128200.

(−)-5′α-Bromo-2′-demethoxygriseofulvin (12)——**12** was prepared from **10** under the same conditions as described for the preparation of **8** from **6**. A mixture of **10** (462 mg) and $PyHBr_3$ (551 mg) in chloroform (12 ml) was refluxed for 2 h. The reaction mixture was poured into a large volume of ice and water, and extracted with chloroform (50 ml×3). The chloroform extract was washed with water, dried (Na_2SO_4) of the two products (**11** and **12**) was determined by gas liquid chromatography. The mixture was separated by repeated recrystallization from methanol and then silica gel column chromatography. Recrystallization of the product from methanol gave (−)-5′α-bromo-2′-demethoxygriseofulvin (**12**) as colorless needles, mp 242—243°C, $[\alpha]_D^{25}$ −435.0° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{14}BrClO_5$: C, 47.84; H, 3.51. Found: C, 47.20; H, 3.67. ^{1}H-NMR and MS were identical with those of **8**. The molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{352}$ −380, $[\theta]_{335}$ −27030, $[\theta]_{321}$ −49840, $[\theta]_{310}$ −46600, $[\theta]_{277}$ 0, $[\theta]_{267}$ +7720, $[\theta]_{259}$ 0, $[\theta]_{253}$ −4380, $[\theta]_{247}$ −2590, $[\theta]_{232}$ −153700, $[\theta]_{230}$ −72740. The mother liquor was chromatographed over silica gel and eluted with benzene–methylene chloride (60: 40). The eluate gave colorless needles of (−)-3′-bromo-2′-demethoxygriseofulvin (**11**) which were recrystallized from methanol, mp 209—211°C, $[\alpha]_D^{25}$ −325.2° (c= 0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{14}BrClO_5$: C, 47.84; H, 3.51. Found: C, 47.72; H, 3.46. ^{1}H-NMR and MS were identical with those of **7**.

(−)-2′-Demethoxydehydrogriseofulvin (3c)——**3c** was obtained from **12** under the same conditions as described for the preparation of **9**. A solution of **12** (107 mg), LiCl (11.4 mg), Li_2CO_3 (20.0 mg) and pyridine (1 ml) in DMF (8.5 ml) was heated at 100°C for 12 h. Then, the reaction mixture was poured into ice and water, and extracted with chloroform (80 ml). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 56.1 mg). The residue was chromatographed on silica gel (20 g) and eluted with benzene–methylene chloride (50: 50). The eluate gave colorless needles of (−)-2′-demethoxydehydrogriseofulvin (**3c**) which were recrystallized from benzene, mp 248—249°C, $[\alpha]_D^{25}$ −31.6° (c=0.20, acetone). *Anal.* Calcd for $C_{16}H_{13}ClO_5$: C, 59.92; H, 4.09. Found: C, 60.36; H, 4.06. ^{1}H-NMR and MS were identical with those of **1c**. Molecular ellipticity $[\theta]$ (c=1.0 mg): $[\theta]_{370}$ +300, $[\theta]_{343}$ +3110, $[\theta]_{327}$ 0, $[\theta]_{300}$ −8890, $[\theta]_{282}$ 0, $[\theta]_{270}$ +5480, $[\theta]_{263}$ 0, $[\theta]_{255}$ −6070, $[\theta]_{248}$ −3560, $[\theta]_{238}$ −40890.

(+)-2′-Demethoxy-2′,3′-dihydrogriseofulvin (13)——**13** was prepared by catalytic hydrogenation of **6**.[12] Recrystallization from methanol gave colorless needles, mp 186—187°C (lit.,[12] 168—170°C), $[\alpha]_D^{24}$ +44.3° (c=0.19, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{17}ClO_5$: C, 59.17; H, 5.28. Found: C, 59.66; H, 5.30. MS m/z: 324 (M^+) (for ^{35}Cl-compound, base peak), 309, 255, 241, 215. ^{1}H-NMR δ (ppm): 0.94 (3H, d, J=6.4 Hz, 6′β-Me), 2.18—2.28 (2H, m, 2′α- and 2′β-H), 2.45—2.55 (3H, m, 3′α-, 5′α- and 6′α-H), 2.91 (1H, dd, J=16.0 and 12.0 Hz, 5′β-H), 3.15 (1H, m, 3′β-H), 3.98 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.14 (1H, s, 5-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{354}$ 0, $[\theta]_{340}$ −1620, $[\theta]_{333}$ −1300, $[\theta]_{328}$ −1430, $[\theta]_{318}$ 0, $[\theta]_{290}$ +10370, $[\theta]_{250}$ +490, $[\theta]_{235}$ +13280, $[\theta]_{230}$ +9720.

(−)-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (14)——A solution of **13** (1.3 g) and selenium dioxide (1.3 g) in *tert*-butanol (40 ml) was refluxed for 12 h. The ratio (36: 64, **14** and **6**) of the two products was determined by gas liquid chromatography. After removal of the solvent under reduced pressure and extraction of the residue with benzene, the benzene extract was directly subjected to column chromatography over silica gel (80 g). 1) Elution with benzene–methylene chloride (40: 60) and recrystallization of the product from methanol gave (+)-2′-demethoxygriseofulvin (**6**) as colorless needles, mp 182—183°C. 2) Elution with benzene–methylene chloride (30: 70) and recrystallization of the product from methanol gave (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**14**) as colorless needles, mp 221—222°C, $[\alpha]_D^{24}$ −211.0° (c=0.10 $CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 58.94; H, 4.67. MS m/z: 322 (M^+) (for ^{35}Cl-compound), 294, 214 (base peak). ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.32 (1H, ddd, $J_{2'\beta-2'\alpha}$=13.6, $J_{2'\beta-3'\alpha}$=5.6, and $J_{2'\beta-3'\beta}$=5.4 Hz, 2′β-H), 2.46 (1H, ddd, $J_{2'\alpha-2'\beta}$= 13.6, $J_{2'\alpha-3'\beta}$=11.0, and $J_{2'\alpha-3'\alpha}$=5.6 Hz, 2′α-H), 2.58 (1H, ddd, $J_{3'\alpha-3'\beta}$=17.1, $J_{3'\alpha-2'\alpha}$=5.6, and $J_{3'\alpha-2'\beta}$=5.6 Hz, 3′α-H), 2.98 (1H, ddd, $J_{3'\beta-3'\alpha}$=17.1, $J_{3'\beta-2'\alpha}$=11.0, and $J_{3'\beta-2'\beta}$=5.4 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (1H, s, 5′-H), 6.15 (1H, s, 5-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ −190, $[\theta]_{336}$ −21670, $[\theta]_{333}$ −21410, $[\theta]_{322}$ −31560, $[\theta]_{311}$ −26890, $[\theta]_{291}$ 0, $[\theta]_{264}$ +17710, $[\theta]_{242}$ 0, $[\theta]_{234}$ −128800.

(+)-2′-Demethoxy-2′,3′-dihydroepigriseofulvin (15)——A suspension of 5% palladium charcoal catalyst (100 mg) in an ethyl acetate solution (100 mg) of **14** (270 mg) was shaken under a stream of hydrogen at atmospheric pressure and at room temperature for 4 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The ratio (47: 53) of the products (**15** and **13**) was determined by gas liquid chromatography. The crude product (98 mg) in benzene was chromatographed on silica gel (20 g). 1) Elution with benzene–methylene chloride (50: 50) gave (+)-2′-demethoxy-2′,3′-dihydroepigriseofulvin (**15**) as an amorphous powder, $[\alpha]_D^{21}$ +15.2° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{17}ClO_5$: C, 59.17; H, 5.28. Found: C, 59.30; H, 5.36. MS m/z: 324 (M^+) (for ^{35}Cl-compound, base peak), 309, 255, 241, 215. ^{1}H-NMR δ (ppm): 0.81 (3H, d, J=6.4 Hz, 6′α-Me), 3.98 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.13 (1H, s, 5-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{354}$ 0, $[\theta]_{337}$ −4750, $[\theta]_{331}$ −4110, $[\theta]_{305}$ 0, $[\theta]_{284}$ +10330, $[\theta]_{281}$ +10060, $[\theta]_{279}$ +10190, $[\theta]_{249}$ +670, $[\theta]_{230}$ +13630. 2) Elution with benzene–methylene chloride (40: 60) and recrystallization of the product from methanol gave (+)-2′-demethoxy-2′,3′-dihydrogriseofulvin (**13**).

(+)-2′-Demethoxyepigriseofulvin (16)——A solution of **15** (50 mg) and selenium dioxide (50 mg) in *tert*-butanol (8 ml) was refluxed for 12 h. The ratio (50: 50, **16** and **14**) of the two products was determined by gas liquid chromatography. After removal of the solvent under reduced pressure, the residue was taken up in benzene and the solution was directly subjected to column chromatography over silica gel (20 g). 1) Elution with benzene–methylene chloride (50: 50) and recrystallization of the product from methanol gave (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**14**). 2) Elution with benzene–methylene chloride (40: 60) and recrystallization of the product afforded (+)-2′-demethoxyepigriseofulvin (**16**), as colorless needles, mp 170—171°C, $[\alpha]_D^{20}$ +145.2° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 60.21; H, 4.77. MS m/z: 322 (M^+) (for ^{35}Cl-compound), 214 (base peak). ^{1}H-NMR δ (ppm): 1.02 (3H, d, J=6.2 Hz, 6′α-Me), 2.58 (1H, dd, J=16.0 and 10.0 Hz, 5′α-H), 2.72 (1H, m, 6′β-H), 2.82 (1H, dd, J=16.0 and 4.0 Hz, 5′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.14 (1H, s, 5-H), 6.27 (1H, d, J=10.2 Hz, 3′-H), 6.48 (1H, d, J=10.2 Hz, 2′-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{375}$ +490, $[\theta]_{336}$ +16590, $[\theta]_{333}$ +13140, $[\theta]_{322}$ +22250, $[\theta]_{291}$ 0, $[\theta]_{269}$ −10940, $[\theta]_{248}$ −1910, $[\theta]_{240}$ 0, $[\theta]_{232}$ +37810.

Dihydroepigriseofulvin (19)——A suspension of 5% palladium-charcoal catalyst (200 mg) in an ethyl acetate solution (200 ml) of (+)-epigriseofulvin (**20**) (500 mg) was shaken under a stream of hydrogen at atmospheric pressure and at room temperature. The hydrogenation was stopped after 2 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The ratio of the product and starting material was determined to be 80: 20 by gas liquid chromatography. The crude product (541 mg) in benzene was chromatographed on silica gel (60 g). Elution with benzene–methylene chloride (60: 40) and recrystallization of the product from methanol gave (+)-dihydroepigriseofulvin (**19**) as colorless needles, mp 186—187°C, $[\alpha]_D^{18}$ +0.2° (c=0.63, acetone). *Anal.* Calcd for $C_{17}H_{19}ClO_6$: C, 57.55; H, 5.40. Found: C, 57.08; H, 5.47. MS$m/z$: 354 (M^+)(for ^{35}Cl-compound), 322, 255 (base peak). ^{1}H-NMR δ (ppm): 0.82 (3H, d, J=6.2 Hz, 6′α-Me), 2.3—2.4 (2H, m, 5′β- and 6′β-H), 2.62 (1H, dd, J=14.5 and 14.5 Hz, 5′α-H), 2.85—2.90 (2H, m, 3′α- and 3′β-H), 3.22 (3H, s, 2′-OMe), 3.86 (1H, dd, J=12.0 and 7.0 Hz, 2′β-H), 4.02 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.14 (1H, s, 5-H). Molecular ellipticity $[\theta]$ c=1.0 mg/ml): $[\theta]_{360}$ 0, $[\theta]_{336}$ +5560, $[\theta]_{323}$ +6370, $[\theta]_{297}$ 0, $[\theta]_{285}$ −6940, $[\theta]_{249}$ 0, $[\theta]_{240}$ +7080, $[\theta]_{235}$ 0, $[\theta]_{230}$ −10270.

(+)-2′,3′-Dihydrodehydroepigriseofulvin (21)——A mixture of dihydroepigriseofulvin (**19**) (353 mg) and selenium dioxide (350 mg) in *tert*-butanol (15 ml) was refluxed for 16 h. The ratio (76: 24,**23** and **22**) of the products in the reaction mixture was determined by gas liquid chromatography. The hot reaction mixture was filtered and Darco-G-60 (300 mg) was added. The mixture was filtered again after 20 min. After removal of the solvent under reduced pressure and extraction of the residue with benzene, the benzene extract was directly subjected to column chromatography over silica gel (50 g). 1) Elution with benzene–methylene chloride (50: 50) gave (+)-2′,3′-dihydrodehydroepigriseofulvin (**21**) as an amorphous powder, $[\alpha]_D^{24}$ +392.5° (c=0.11, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 57.65; H, 4.85. MS m/z: 352 (M^+) (for ^{35}Cl-compound). ^{1}H-NMR δ (ppm): 1.82 (3H, s, 6′-Me), 2.78 (1H, dd, J=16.0 and 8.0 Hz, 3′β-H), 3.08 (1H, dd, J=16.0 and 4.0 Hz, 3′α-H), 3.38 (3H, s, 2′-OMe), 3.96 (1H, dd, J=6.0 and 4.0 Hz, 2′β-H), 6.12 (1H, s, 5′-H), 6.14 (1H, s, 5-H). The molecular ellipticity $[\theta]$ (c=0.7 mg/ml): $[\theta]_{370}$ +470, $[\theta]_{337}$ +24170, $[\theta]_{333}$ +23960, $[\theta]_{323}$ +29430, $[\theta]_{295}$ 0, $[\theta]_{280}$ −20800, $[\theta]_{273}$ −19190, $[\theta]_{257}$ −22050, $[\theta]_{243}$ 0, $[\theta]_{232}$ +40560. 2) Elution with benzene–methylene chloride (40: 60) gave (+)-epigriseofulvin (**22**).

(−)-2′-Demethoxyepigriseofulvin (17)——**19** (354 mg) was heated under reflux in 2 N sulfuric acid (42 ml) and ethanol (32 ml) for 6 h. The solution was neutralized with 5 N Na_2CO_3, and the solid (197 mg) obtained by concentration of the solution *in vacuo* and filtration was chromatographed on silica gel (20 g). Elution with benzene–methylene chloride (50: 50) afforded a product which was recrystallized from methanol to afford (−)-2′-demethoxyepigriseofulvin (**17**) as colorless needles, mp 171—172°C, $[\alpha]_D^{18}$ −147.1° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 59.56; H, 4.68. ^{1}H-NMR and MS were identical with those of **16**. Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{375}$ −480, $[\theta]_{336}$ −16580, $[\theta]_{333}$ −13170, $[\theta]_{322}$ −22220, $[\theta]_{291}$ +10950, $[\theta]_{248}$ +1930, $[\theta]_{240}$ 0, $[\theta]_{232}$ −37800.

(−)-2′-Demethoxy-2′,3′-dihydroepigriseofulvin (22)——A suspension of 5% palladium charcoal catalyst (200 mg) in ethyl acetate solution (200 ml) of **19** (468 mg) was shaken under a stream of hydrogen at atmospheric pressure and at room temperature for 4 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue (444 mg) was found to show only one peak on gas liquid chromatography. It was subjected to column chromatography over silica gel (30 g) in benzene, and elution with benzene–methylene chloride (50: 50) gave (−)-2′-demethoxy-2′,3′-dihydroepigriseofulvin (**22**) as an amorphous powder $[\alpha]_D^{24}$ −14.4° (c=0.12, $CHCl_3$). *Anal.* Calcd for $C_{16}H_{17}ClO_5$: C, 59.17; H, 5.28. Found: C, 59.25; H, 5.38. ^{1}H-NMR and MS were identical with those of **15**. Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{354}$ 0, $[\theta]_{337}$ +4720, $[\theta]_{331}$ +3960, $[\theta]_{305}$ 0, $[\theta]_{284}$ −11440, $[\theta]_{281}$ −10800, $[\theta]_{279}$ −11520, $[\theta]_{249}$ −800, $[\theta]_{230}$ −12400.

(+)-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (18)——A solution of **22** (440 mg) and selenium dioxide (400 mg) in *tert*-butanol (20 ml) was refluxed for 20 h. The ratio (50: 50, **20** and **19**) of the two products was determined by gas liquid chromatography. After removal of the solvent under reduced pressure, the residue was directly applied to column chromatography over silica gel (50 g) in benzene. Elution with benzene–methylene chloride (30: 70) and recrystallization of the product from methanol gave (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**18**) as colorless needles, mp 210—220°C, $[\alpha]_D^{24}$ +208.3° (c=0.10,

$CHCl_3$). *Anal.* Calcd for $C_{16}H_{15}ClO_5$: C, 59.54; H, 4.68. Found: C, 59.75; H, 4.59. ^{1}H-NMR and MS were identical with those of **14**. Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ +160, $[\theta]_{336}$ +21090, $[\theta]_{333}$ +21570, $[\theta]_{322}$ +30910, $[\theta]_{311}$ +25120, $[\theta]_{291}$ 0, $[\theta]_{264}$ −17550, $[\theta]_{242}$ 0, $[\theta]_{234}$ +116600.

[2′α, 3′α-$^2H_2$]-15 (13a)——**13a** was obtained from **6** (1.2 g) by the same procedures as described for the preparation of **13** from **6** except for the use of deuterium (purity 98%, Nissan Shoji Co., Ltd.), mp 178—180°C. MS: $^2H_0$ 3.0%, $^2H_1$ 16.7%, $^2H_2$ 80.3%. ^{1}H-NMR δ (ppm): 0.94 (3H, d, J=6.4 Hz, 6′β-Me), 2.20 (1.2 H, d, J=6.0 Hz, 2′α- and 2′β-H region), 2.45—2.55 (2.2H, m, 3′α-, 5′α- and 6′α-H), 2.92 (1H, m, 5′β-H), 3.03 (1H, m, 3′β-H), 3.98 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.14 (1H, s, 5-H).

[2′α-^{2}H]-15 (13b)——**13a** (500 mg) was heated under reflux with 2 N sulfuric acid (600 ml) and ethanol (45 ml) for 6 h. The mixture was neutralized with 5 N Na_2CO_3, and the solid (462 mg) obtained by concentration *in vacuo* and filtration was chromatographed on silica gel (50 g). Elution with benzene–methylene chloride (60: 40) gave a product which was recrystallized form methanol to afford **13b** as colorless needles, mp 178—180°C. MS $^2H_0$ 14.5%, $^2H_1$ 85.5%. ^{1}H-NMR δ (ppm): 0.94 (3H, d, J=6.4 Hz, 6′β-Me), 2.20 (1.1H, dd, J=6.0 and 6.0 Hz, 2′β-H), 2.45—2.55 (3H, m, 3′α-, 5′α- and 6′α-H), 2.92 (1H, dd, J=16.0 and 12.0 Hz, 5′β-H), 3.03 (1H, dd, J=16.0 and 7.6 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.14 (1H, s, 5-H).

[2′β-^{2}H]-15 (13c)——**4a** was obtained from **2a** (1.0 g) by the same procedures as described for the preparation of **2** from **4** except for the use of deuterium, mp 218—219°C. MS: $^2H_0$ 33.4%, $^2H_1$ 46.2%, $^2H_2$ 20.4%. The ^{1}H-NMR spectrum was almost the same as that of **4** except for some differences in the signal patterns of 2′α-, 3′α-, 3′β-, and 5′α-H. **6a** was obtained from **4a** by the same procedures as described for the preparation of **6** from **4**, mp 184—185°C. MS: $^2H_0$ 46.1%, $^2H_1$ 53.9%. ^{1}H-NMR δ (ppm): 0.93 (3H, d, J=6.2 Hz, 6′β-Me), 2.45 (1H, dd, J=16.2 and 4.2 Hz, 5′α-H), 2.88 (1H, m, 6′α-H), 3.12 (1H, dd, J=18.0 and 14.0 Hz, 5′β-H), 3.98 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.15 (1H, s, 5-H), 6.19 (1H, d, J=10.0 Hz and s, 3′-H), 6.57 (0.6H, d, J=10.0 Hz, 2′-H). **13c** was obtained from **6a** by the same procedures as described for the preparation of **13** from **6**, mp 178—180°C. MS: $^2H_0$ 51.2%, $^2H_1$ 48.8%. ^{1}H-NMR δ (ppm): 0.94 (3H, d, J=6.4 Hz, 6′β-Me), 2.23 (1.5H, J=8.0 and 5.4 Hz, 2′α- and 2′β-H (1: 0.5, respectively), 2.45—2.55 (3H, m, 3′α-, 5′α- and 6′α-H), 2.92 (1H, dd, J=16.0 and 12.0 Hz, 5′β-H), 3.03 (1H, m, 3′β-H), 3.98 (3H, s, 4-OMe), 4.02 (3H, s, 6-OMe), 6.14 (1H, s, 5-H).

[2′α, 3′α-$^2H_2$]-16 (14a)——**14a** was prepared from **13a** (420 mg) under the same conditions as described for the preparation of **14** from **13**. The ratio (65: 35, **16a** and **6b**) of the products in the reaction was determined by gas liquid chromatography. The reaction mixture was separated under the same conditions as described for the preparation of **14** from **13**. 1) Elution with benzene–methylene chloride (60: 40) and recrystallization of the product from methanol gave colorless needles of **6b**, mp 185—187°C. MS: $^2H_0$ 35.5%, $^2H_1$ 60.9%, $^2H_2$ 3.6%. ^{1}H-NMR δ (ppm): 0.93 (3H, d, J=6.2 Hz, 6′β-Me), 2.45 (1H, dd, J=16.2 and 4.2 Hz, 5′α-H), 2.88 (1H, m, 6′α-H), 3.12 (1H, dd, J=18.0 and 14.0 Hz, 5′β-H), 3.98 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.19 (0.3H, d, J=10.0 Hz, 3′-H), 6.57 (1H, d, J=10.0 Hz and s, 2′-H). 2) Elution with benzene–methylene chloride (30: 70) and recrystallization of the product from methanol gave colorless needles of **14a**, mp 211—212°C. MS: $^2H_0$ 2.7%, $^2H_1$ 9.6%, $^2H_2$ 87.7%. ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.30 (1H, d, J=5.0 Hz, 2′β-H), 2.96 (1H, br d, J=5.0 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-Me), 6.10 (1H, s, 5-H).

[2′α-^{2}H]-16 (14b)——**14b** was prepared from **13b** (400 mg) under the same conditions as described for the preparation of **14** from **13**. The ratio (50: 50, **14b** and **6c**) of the products in the reaction was determined by gas liquid chromatography. The reaction mixture was separated under the same conditions as described for the preparation of **14** from **13**. 1) Elution with benzene–methylene chloride (60: 40) and recrystallization of the product from methanol gave colorless needles of **6c**, mp 184—185°C. MS: $^2H_0$ 96.3%, $^2H_1$ 3.7%. 2) Elution with benzene–methylene chloride (30: 70) and recrystallization of the product from methanol gave colorless needles of **14b**, mp 210—212°C. MS: $^2H_0$ 8.8%, $^2H_1$ 90.2%. ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.30 (1H, dd, J=6.0 and 6.0 Hz, 2′β-H), 2.58 (1H, dd, J=17.0 and 6.0 Hz, 3′α-H), 2.98 (1H, dd, J=16.6 and 6.0 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (1H, s, 5′-H), 6.15 (1H, s, 5-H).

[2′β-^{2}H]-14 (14c)——**14c** was prepared from **13c** (430 mg) under the same conditions as described for the preparation of **14** from **13**. The ratio (50: 50, **14c** and **6d**) of the products was determined by gas liquid chromatography. The reaction mixture was separated under the same conditions as described for the preparation of **14** from **13**. 1) Elution with benzene–methylene chloride (60: 40) and recrystallization of the product from methanol gave colorless needles of **6d**, mp 186—187°C. MS: $^2H_0$ 38.0%, $^2H_1$ 60.6%, $^2H_2$ 1.4%. ^{1}H-NMR δ (ppm): 0.93 (3H, d, J=6.2 Hz, 6′-Me), 2.45 (1H, dd, J=16.2 and 4.2 Hz, 5′α-H), 3.98 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.15 (1H, s, 5-H), 6.19 (1H, d, J=10.0 Hz and s, 3′-H), 6.57 (0.4H, d, J=10.0 Hz, 2′-H). 2) Elution with benzene–methylene chloride (60: 40) and recrystallization of the product from methanol gave colorless needles of **14c**, mp 210—212°C. MS: $^2H_0$ 37.6%, $^2H_1$ 60.7%. ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.30 (0.5H, m, 2′β-H), 2.42 (1H, m, 2′α-H), 2.57 (1H, dd, J=16.4 and 6.0 Hz, 3′α-H), 2.96 (1H, m, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (1H, s, 5′-H), 6.15 (1H, s, 5-H).

Microbial Transformation of (+)-2′-Demethoxydehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——All of the experiments were essentially the same as those described in the previous paper[2] except that (+)-2′-demethoxydehydrogriseofulvin (**1c**) was used as the substrate. The time course of the

incubation is shown in Fig. 5. 1) Column chromatography of the residue from the incubation supernatant (after 12 h) on silica gel and recrystallization of the products from methanol gave a 81: 19 mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin, and (+)-2′-demethoxygriseofulvin. A mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin: mp 217—219°C, $[\alpha]_D^{22}$ +131.6° (c=0.10, $CHCl_3$). MS m/z: 322 (M^+) (for ^{35}Cl-compound), 294, 214 (base peak). ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.32 (1H, ddd, $J_{2'\beta-2'\alpha}$=13.6, $J_{2'\beta-2'\alpha}$=5.6, and $J_{2'\beta-3'\beta}$=5.4 Hz, 2′β-H), 2.46 (1H, ddd, $J_{2'\alpha-2'\beta}$=13.6, $J_{2'\alpha-3'\beta}$=11.0, and $J_{2'\alpha-3'\alpha}$=5.6 Hz, 2′α-H), 2.58 (1H, ddd, $J_{3'\alpha-3'\beta}$=17.1, $J_{3'\alpha-2'\alpha}$=5.6, and $J_{3'\alpha-2'\beta}$=5.6 Hz, 3′α-H), 2.98 (1H, ddd, $J_{3'\beta-3'\alpha}$=17.1, $J_{3'\beta-2'\alpha}$=11.0 and $J_{3'\beta-2'\beta}$=5.4 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (1H, s, 5′-H), 6.15 (1H, s, 5-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ 0, $[\theta]_{336}$ +12940, $[\theta]_{333}$ +12690, $[\theta]_{322}$ +18890, $[\theta]_{311}$ +16100, $[\theta]_{291}$ 0, $[\theta]_{264}$ −700, $[\theta]_{242}$ 0, $[\theta]_{234}$ +40870. (+)-2′-Demethoxygriseofulvin: mp 220—221°C, $[\alpha]_D^{20}$ +408.0° (c=0.09, $CHCl_3$). MS m/z: 322 (M^+) (for ^{35}Cl-compound), 214 (base peak). ^{1}H-NMR δ (ppm): 0.93 (3H, d, J=6.2 Hz, 6′β-Me), 2.45 (1H, dd, J=16.2 and 4.2 Hz, 5′α-H), 2.88 (1H, m, 6′α-H), 3.12 (1H, dd, J=16.2 and 14.0 Hz, 5′β-H), 3.98 (3H, s, 4-OMe), 4.04 (3H, s, 6-OMe), 6.15 (1H, s, 5-H), 6.19 (1H, d, J=10.0 Hz, 3′-H), 6.57 (1H, d, J=10.0 Hz, 2′-H). Molecular ellipticity $[\theta]$ (c=0.9 mg/ml): $[\theta]_{376}$ 0, $[\theta]_{354}$ −2050, $[\theta]_{349}$ 0, $[\theta]_{337}$ +17400, $[\theta]_{336}$ +17310, $[\theta]_{322}$ +37550, $[\theta]_{316}$ +35400, $[\theta]_{312}$ +36740, $[\theta]_{274}$ 0, $[\theta]_{247}$ −18240, $[\theta]_{241}$ 0, $[\theta]_{230}$ +128910. 2) Column chromatography of the residue from the incubation supernatant (after 3 h) on silica gel and recrystallization of the products from methanol gave a 72: 28 mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin, (+)-2′-demethoxygriseofulvin and (+)-2′-demethoxydehydrogriseofulvin. A mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin: mp 218—219°C. Molecular ellipticity $[\theta]$ (c=0.8 mg/ml): $[\theta]_{370}$ 0, $[\theta]_{336}$ +9580, $[\theta]_{333}$ +9380, $[\theta]_{322}$ +14180, $[\theta]_{311}$ +12230, $[\theta]_{291}$ 0, $[\theta]_{264}$ −680, $[\theta]_{234}$ +30980. MS and ^{1}H-NMR spectra were identical with those of the standard samples (**16** or **20**). (+)-2′-Demethoxygriseofulvin: mp 220—221°C. MS, ^{1}H-NMR and CD were identical with those of the standard sample (**6**). (+)-2′-Demethoxydehydrogriseofulvin: mp 247—248°C, $[\alpha]_D^{24}$ +30.3° (c=0.20, acetone). MS m/z: 320 (M^+) (for ^{35}Cl-compound, base peak), 281, 291, 274, 197, 140. ^{1}H-NMR δ (ppm): 1.82 (3H, d, J=2.0 Hz, 6′-Me), 3.98 (3H, s, 4-OMe), 4.05 (3H, s, 6-OMe), 6.18 (1H, s, 5-H), 6.29 (1H, br s, 5′-H), 6.41 (1H, d, J=10.0 Hz, 3′-H), 6.55 (1H, d, J=10.0 Hz, 2′-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ −280, $[\theta]_{343}$ −3380, $[\theta]_{327}$ 0, $[\theta]_{300}$ +9010, $[\theta]_{284}$ 0, $[\theta]_{270}$ −5620, $[\theta]_{263}$ 0, $[\theta]_{255}$ +6300, $[\theta]_{248}$ +3600, $[\theta]_{238}$ +40210.

Microbial Transformation of (−)-2′-Demethoxydehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——All of the experiments were essentially the same as those described in the previous paper[2] except that (−)-2′-demethoxydehydrogriseofulvin (**3c**) was used as the substrate. The time course of the incubation is shown in Fig. 5. Column chromatography of the residue from the incubation supernatant (after 12 h) on silica gel and recrystallization from benzene gave (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin, mp 216—217°C, $[\alpha]_D^{22}$ +212.1° (c=0.12, $CHCl_3$). MS m/z: 322 (M^+) (for ^{35}Cl-compound), 294, 214 (base peak). ^{1}H-NMR δ (ppm): 1.79 (3H, d, J=1.8 Hz, 6′-Me), 2.32 (1H, ddd, $J_{2'\beta-2'\alpha}$=13.6, $J_{2'\beta-3'\alpha}$=5.6, and $J_{2'\beta-3'\beta}$=5.4 Hz, 2′β-H), 2.46 (1H, ddd, $J_{2'\alpha-2'\beta}$=13.6, $J_{2'\alpha-3'\beta}$=11.0, and $J_{2'\alpha-3'\alpha}$=5.6 Hz, 2′α-H), 2.58 (1H, ddd, $J_{3'\alpha-3'\beta}$=17.1, $J_{3'\alpha-2'\alpha}$=5.6, and $J_{3'\alpha-2'\beta}$=5.6 Hz, 3′α-H), 2.98 (1H, ddd, $J_{3'\beta-2'\alpha}$=17.1, $J_{3'\beta-2'\alpha}$=11.0, and $J_{3'\beta-2'\beta}$=5.4 Hz, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (1H, s, 5′-H), 6.15 (1H, s, 5-H). Molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ +170, $[\theta]_{336}$ +21110, $[\theta]_{333}$ +21430, $[\theta]_{322}$ +30950, $[\theta]_{311}$ +25150, $[\theta]_{291}$ 0, $[\theta]_{264}$ −17570, $[\theta]_{242}$ 0, $[\theta]_{234}$ +116680.

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