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Microbial Transformation of (+)- and (−)-Dehydrogriseofulvin by *Streptomyces* Species Analyzed by ^2H Nuclear Magnetic Resonance Spectroscopy

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To elucidate the stereochemical course of the microbial transformation of (+)-dehydrogriseofulvin (DGF) by *Streptomyces* species, (+)-[5′-^{2}H]DGF, which was obtained by dehydrogenation of (+)-[5′α,5′β-^{2}H]epigriseofulvin, was incubated with *S. cinereocrocatus*. The structure of the product was identified as (+)-[5′α-^{2}H]griseofulvin (GF) by ^2H nuclear magnetic resonance spectroscopy. The results of incubations of (−)- and (+)-DGF with eight *Streptomyces* species (*S. cinereocrocatus*, *S. roseochromogenus*, *S. bikiniensis*, *S. griseinus*, *S. durhamensis*, *S. californicus*, *S. fimbriatus*, and *S. cinereoruber*) showed that (−)- and (+)-DGF were isomerized to each other and converted to (+)-GF as the main product, although some (−)-GF was also formed depending on the microorganisms used. In particular, incubation of (+)-DGF with *S. cinereoruber* yielded (−)-GF as the main product. In addition, (±)-DGF was transformed by *S. cinereocrocatus* to the naturally occurring (+)-GF in 48% yield. The enantiomer ratios of DGF and GF obtained by microbial transformation were calculated from the $[\alpha]_D$ values of corresponding enantiomer mixtures.

Keywords——microbial transformation; griseofulvin; ^2H NMR; CD; optical rotation; isomerization; enantiomer; *Streptomyces* species

Introduction

The importance of microbial transformation of natural products has been emphasized in many excellent reviews.[1] The microbial transformation of (−)-dehydrogriseofulvin (DGF) (**1a**)[2] to (+)-griseofulvin (GF) (**2a**) was initially investigated by Andres and his co-workers[3] using *Streptomyces cinereocrocatus* NRRL 3443. Previously, we deduced by means of ^2H NMR spectroscopy[4,5] the stereochemical course[6] of the microbial hydrogenation of natural (−)-[5′-^{2}H]DGF (**1b**) into natural (+)-[5′α-^{2}H]GF (**2d**) by the same microorganism.

This paper describes the results of incubations of (−)- and (+)-DGF with eight *Streptomyces* species; (−)- and (+)-DGF were isomerized to each other and hydrogenated to (+)-GF as the main product. In this work, optical rotation and ^2H nuclear magnetic resonance (NMR) measurements were utilized to demonstrate the isomerization of DGF and the stereochemistry of GF formation, respectively.

Results and Discussion

Syntheses of Substrates and Related Compounds

Griseofulvin derivatives were obtained from natural (+)-GF. (−)-DGF (**1a**) (Chart 1) was prepared according to the method of Taub *et al.*,[2c] and (+)-epigriseofulvin (**3a**) (Chart 2) was synthesized from (+)-GF by the procedure of MacMillan.[7a] The enantiomer (**4a**) of (−)-DGF was prepared by dehydrogenation of **3a** with selenium dioxide in boiling *tert*-butanol. The product (**4a**) was identical with (−)-DGF as judged from the ^1H NMR and mass spectral data, but its circular dichroism (CD) (Fig. 1) and optical rotation ($[\alpha]_D^{19}$ +35.2°) data were different. Thus, the structure of **4a** is (+)-DGF. (±)-DGF[2c,7a] was obtained by dehydrogenation of (+)-GF with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (Merck–Schuchardt)

in dioxane solution. Catalytic hydrogenation of **4a** was performed essentially by the procedure of Kuo *et al.*[8a] The ^{1}H NMR and mass spectra of the product (**5**) were identical with those of (+)-griseofulvin. However, the CD (Fig. 2) and optical rotation ($[\alpha]_D^{21}$ −338.2°), together with the above data, clearly indicated that **5** is (−)-GF,[2c,8] which is the enantiomer of natural (+)-GF.

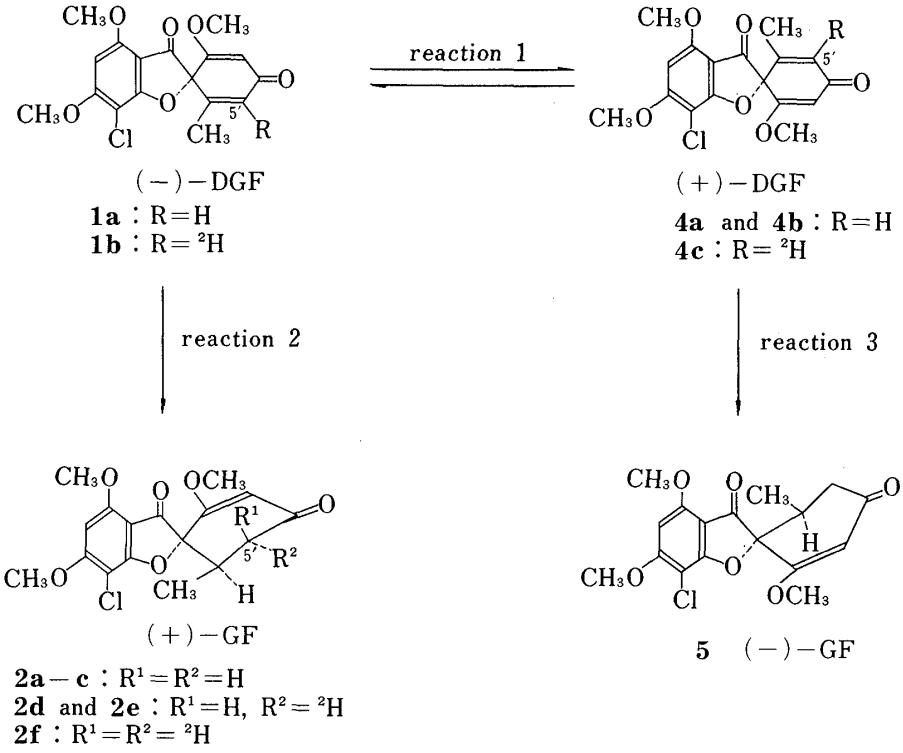

Chart 1

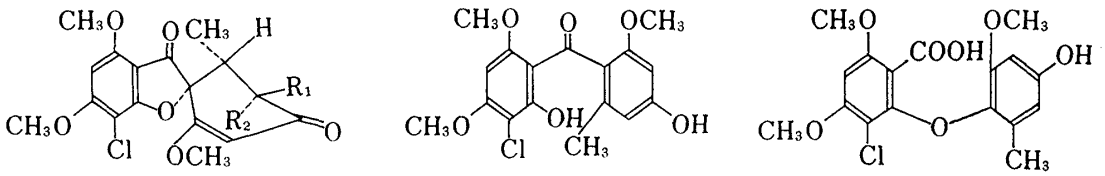

Chart 2

Further, (+)-[5′-^{2}H]DGF (**4c**) was prepared by dehydrogenation of (+)-[5′α,5′β-^{2}H]-epigriseofulvin (**3b**) to serve as a partially deuterated substrate in the fermentation experiment (see Fig. 3 for ^{2}H NMR of **4c**).

In order to allow the unambiguous assignment of 4- and 6-OCH_3 signals in the ^{1}H NMR spectra of the griseofulvin derivatives, (+)-GF, (−)-DGF, and (+)-epigriseofulvin deuterated at the 4-methoxy group were synthesized. The deuterated derivative of (+)-GF (0.69 ^{2}H/molecule from mass spectrometric analysis) was obtained by methylation of 4-demethylgriseofulvin with deuterated diazomethane. Subsequent treatment of the (+)-[4-OCH_3-^{2}H]GF with selenium dioxide afforded the corresponding deuterated dehydro-derivative, (−)-DGF (0.66 ^{2}H/molecule). Deuterated (+)-epigriseofulvin (1.12 ^{2}H/molecule) was obtained by treatment of 4-demethylepigriseofulvin with deuterated diazomethane. Comparison

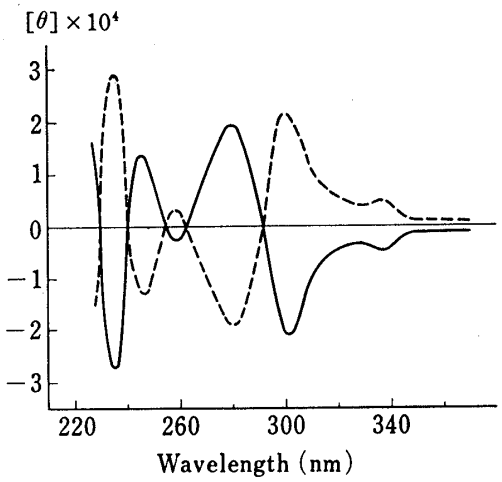

Fig. 1. CD Spectra of (−)- and (+)-Dehydrogriseofulvin (**1a** and **4a**)

——: for **1a**; ----: for **4a**.

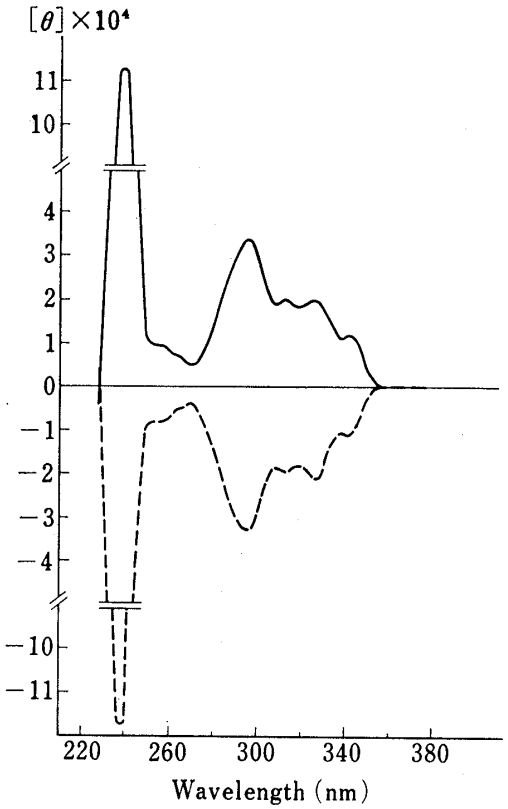

Fig. 2. CD Spectra of (+)- and (−)-Griseofulvin (**2a** and **5**)

——: for **2a**; ----: for **5**.

of the ^1H NMR spectra of these pairs of deuterated and undeuterated compounds revealed that ^1H signals of 4-OCH_3 appear at higher field than those of 6-OCH_3 in the three cases.

Microbial Transformation of (−)- and (+)-Dehydrogriseofulvin by *Streptomyces cinereocrocatus*

(+)-DGF (**4a**) was administered to a shaken culture of *S. cinereocrocatus* on the 4th day of the fermentation as described previously.[6] After 3 days, GF (**2b**) was obtained in 35—45% yields from the broth, and DGF (**4b**) was recovered in 10—15% yields. The structure of **2b**, mp 228—229°C, $[\alpha]_D^{21}$ +336.9°, which was inferred from ^1H NMR and mass spectral data together with gas liquid chromatography (GLC) and thin-layer chromatography (TLC) data, was confirmed to be (+)-GF by comparison of its CD spectrum with those of natural (+)-GF and (−)-GF (**5**). The recovered **4b**, on the other hand, was pure (+)-DGF without any contamination by (−)-DGF in spite of the fermentation conditions under which (+)-GF was formed. When the incubation period was shortened by one day, on the other hand, GF (**2b**) and DGF (**4b**) were obtained in 12 and 40% yields, respectively.

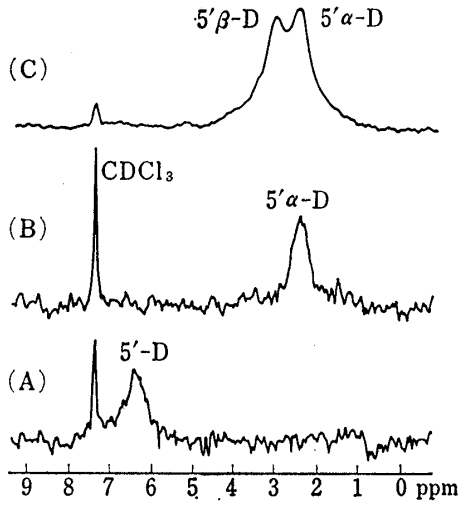

Fig. 3. ^2H NMR Spectra of Deuterated Griseofulvin Derivatives

(A): **4c**, 10 mg, 2200 transients.
(B): **2e**, 8 mg, 6400 transients.
(C): **2f**, 22 mg, 706 transients.

Further, the recovered **4b** was pure (+)-DGF, as described above. Subsequently, the fermentation of *S. cinereocrocatus* with (±)-DGF under the same conditions yielded (+)-GF (**2c**) in 48% yield together with DGF in 8% yield.

The results mentioned above suggest that during the microbial transformation, (+)-DGF (**4a**) is converted first to its enantiomer, natural (−)-DGF (**1a**), followed by hydrogenation of the 5′—6′ double bond to yield natural (+)-GF (**2b**). Consequently, we attempted to analyze the stereochemical course of the hydrogenation of (+)-DGF (**4a**) by S. *cinereocrocatus*. For such a purpose, it has been demonstrated that ^2H NMR spectroscopy[5,6] is a very powerful tool to determine the fate and location of hydrogens during biosynthesis. Therefore, (+)-[5′-^{2}H]DGF (**4c**) was subjected to microbial trasformation by *S. cinereocrocatus* under the same conditions as described above for the undeuterated substrate (**4a**). After an additional 3 days, the cultures were harvested and deuterium-labeled GF was extracted, followed by column chromatographic separation to give 14 mg of deuterated GF, mp 229—230°C ($^2H_0$ 57.4, $^2H_1$ 42.6%) (**2e**). The ^2H NMR spectrum of **2e** was compared with that of (+)-[5′α, 5′β-^{2}H]GF (**2f**) as described previously,[6] and the configuration of the deuterium was unequivocally ascribed as 5′α, establishing the structure of **2e** as (+)-[5′α-^{2}H]GF (see Fig. 3).

As alternative intermediates in the formation of (+)-[5′α-^{2}H]GF, (+)-epigriseofulvin (**3a**), griseophenone A (**6**)[2c,9] and diphenylethercarboxylic acid (**7**)[10] can be considered (Chart 2). The reasons are as follows: (1) **3a** has been chemically interconverted[7a] with (+)-GF; (2) many papers have shown that **6** is transformed into DGF by oxidative coupling by oxidizing reagents,[2b] micro-organisms,[11] horseradish peroxide–H_2O_2 system,[12] and homogenized potato peelings;[13] and (3) **7** is a compound corresponding to (−)-DGF with an opened B-ring. The fermentation of these compounds by *S. cinereocrocatus*, however, did not lead to the formation of GF and/or DGF. These results support the isomerization by microorganism of (+)-DGF to (−)-DGF (**1a**) which then undergoes hydrogenation to give natural (+)-GF.

Microbial Transformation of (−)- and (+)-DGF by Seven *Streptomyces* species

The above results prompted us to investigate the microbial transformation of DGF's

Table I. Relative Ratios of Enantiomers of GF and Those of Recovered DGF in the Microbial Transformation of (−)- and (+)- DGF by *Streptomyces* species

Streptomyces species	Relative ratio (%)[a] of (+)- and (−)-GF from				Relative ratio (%)[b] of (+)- and (−)-DGF from			
	(−)-DGF		(+)-DGF		(−)-DGF		(+)-DGF	
	(+)-GF	(−)-GF	(+)-GF	(−)-GF	(+)-DGF	(−)-DGF	(+)-DGF	(−)-DGF
S. cinereocrocatus	100 (97)[c]	0 (3)[c]	100	0	— (17)[c]	— (83)[c]	99	1
S. roseohromogenus	100 (100)	0 (0)	100	0	— (31)	— (69)	100	0
S. bikiniensis	100 (100)	0 (0)	97	3	— (22)	— (78)	78	22
S. griseinus	98	2	98	2	42	58	67	33
S. durhamensis	98	2	99	1	40	60	86	14
S. californicus	99	1	99	1	60	40	74	26
S. fimbriatus	100	0	91	9	42	58	76	24
S. cinereoruber	88	12	13	87	50	50	44	56

a) $[\alpha]_D^{19}$ values of standard samples of (+)- and (−)-GF were +335.8° and −338.2° in $CHCl_3$, respectively, and for the calculation of the ratios $[\alpha]_D^{19}$ values of +337° and −337° were used as those of (+)- and (−)-GF, respectively.

b) $[\alpha]_D^{19}$ values of standard samples of (−)- and (+)-DGF were −35.6° and +35.2° in acetone, respectively, and for the calculation of the ratios $[\alpha]_D^{19}$ values of −35.4° and +35.4° were used as those of (−)- and (+)-DGF, respectively.

c) The values in parenthese are those of only 1 day compared with 3 days in the standard fermentation. These experiments were done to obtain a much higher recovery of DGF.

by other *Streptomyces* species. *Streptomyces roseochromogenus* (Jensen) Waksman and Henrici (IFO 13080), *Streptomyces bikiniensis* Johnstone and Waksman (IFO 13350), *Streptomyces griseinus* Waksman (IFO 12869), *Streptomyces durhamensis* Gordon and Lapa (IFO 13441), and *Streptomyces californicus* (Waksman and Curtis) Waksman and Henrici (IFO 12750) transformed (−)- and (+)-DGF into (+)-GF in almost the same way as *Streptomyces cinereocrocatus* NRRL 3443. However, in the fermentation by *Streptomyces fimbriatus* (Millard and Burr) Waksman (IFO 13549), some differences were noted, as shown in the tables. The data shown in Tables I, II, and III were obtained after incubation for 3 days following administration of the substrates, except for the additional data (one-day incubation) for *S. cinereocrocatus*. When incubation with the microorganism was prolonged to 5 or 7 days, (+)-GF formed was further transformed into 5′α-hydroxygriseofulvin[3,6] by hydroxylation as described previously. Therefore, we performed the present experiments with *Streptomyces* species under the same conditions (incubation for 3 days) as were used for *S. cinereocrocatus*. During the course of the screening test, although the yield of GF was very low, *Streptomyces cinereoruber* Corbaz *et al.* (IFO 12756) was found to be very different from the *Streptomyces* species described above. When (−)-DGF was incubated with *S. cinereoruber*, GF ($[\alpha]_D^{19}$ +254.5°) was obtained, corresponding to a mixture consisting of 88% (+)-GF and 12% (−)-GF (**5**) on the basis of

Table II. Yields and Physical Data of GF obtained by Microbial Transformation of (−)- and (+)-DGF

Streptomyces species	Yield (%)		mp (°C)		$[\alpha]_D^{19}$ ($CHCl_3$)	
	(−)-DGF[a]	(+)-DGF[b]	(−)-DGF[a]	(+)-DGF[b]	(−)-DGF[a]	(+)-DGF[b]
S. cinereocrocatus	70 (20)[c]	40	228—229 (221—222)[c]	226—227	+334.2 (+314.6)[c]	+336.9
S. roseochromogenus	70 (26)	25	225—227 (223—224)	223—224	+336.1 (+339.3)	+336.2
S. bikiniensis	25 (6)	10	223—224 (221—222)	221—222	+338.8 (+338.5)	+314.4
S. griseinus	37	9	220—222	219—220	+323.5	+326.3
S. durhamensis	31	16	222—226	218—220	+323.2	+328.7
S. californicus	52	19	228—229	227—228	+328.9	+328.9
S. fimbriatus	34	8	223—224	220—221	+338.8	+274.3
S. cinereoruber	8	10	227—228	227—228	+254.5	−250.0

a), *b*) The substrate for the microbial transformation.
c) Values as noted in *c*) of Table I.

Table III. Yields and Physical Data of DGF obtained by Microbial Transformation of (−)- and (+)-DGF

Streptomyces species	Yield (%)		mp (°C)		$[\alpha]_D^{19}$ (acetone)	
	(−)-DGF[a]	(+)-DGF[b]	(−)-DGF[a]	(+)-DGF[b]	(−)-DGF[a]	(+)-DGF[b]
S. cinereocrocatus	0 (37)[c]	13	— (276—288)[c]	270—278	— (−23.4)[c]	+34.8
S. roseochromogenus	8 (22)	28	— (283—285)	270—272	— (−13.2)	+35.4
S. bikiniensis	12 (19)	8	— (282—284)	283—284	— (−19.6)	+19.5
S. griseinus	34	46	287—290	285—290	−5.5	+11.7
S. durhamensis	44	24	285—290	280—281	−6.9	+25.3
S. californicus	18	19	283—287	283—285	+7.3	+17.0
S. fimbriatus	44	83	280—285	275—277	−5.5	+27.2
S. cinereoruber	29	36	294—296	295—297	0	−4.0

a), *b*), *c*) The same as in Table II.

optical activities. When (+)-DGF was incubated under the same conditions, an enantiomer mixture of GF consisting of 87% (−)-enantiomer and 13% (+)-enantiomer was produced.

The above results indicate that during the microbial process (−)- and (+)-DGF were interconverted, followed by hydrogenation at the 5′—6′ double bond to give product(s) corresponding to (+)- or/and (−)-GF (Chart 1).

Conclusions

When (−)-DGF and (+)-DGF were incubated with *Streptomyces* species, mutual interconversion (reaction 1 in Chart 1) occurred, although in the fermentation of (+)-DGF with *S. cinereocrocatus* and *S. roseochromogenus* (+)-DGF was recovered without any contamination by (−)-DGF. The results presented here led us to propose a scheme for the transformation of (−)- and (+)-DGF by *Streptomyces* species (Chart 1). The processes comprise isomerization between (−)- and (+)-DGF and hydrogenation to the corresponding (+)- and (−)-GF, respectively. Except for *S. cinereoruber* which shows almost the same hydrogenation activities in both 1→2 (reaction 2) and 4→5 (reaction 3) processes, other *Streptomyces* species showed very much higher activities in reaction 2 than in reaction 3. Chemical isomerization of DGF has been reported by MacMillan[7a] in 1959. Although the microbial transformation[1] may be brought about by constitutive or substrate-induced enzymes or as yet unknown enzymes, the results mentioned above indicate that the microbial transformation of (−)- and (+)-DGF by *Streptomyces* species is a new type of isomerization to afford the corresponding enantiomer. Using some microbial systems it is possible to obtain natural (+)-GF both directly and stereospecifically from (±)-DGF, which is an important intermediate[2c,8a,8d,8e,8g] in the total synthesis[8,10] of (±)-GF.

Experimental

All melting points were obtained on a micro-melting point apparatus, type MM2 (Shimadzu Seisakusho Ltd.), and are uncorrected. Gas chromatography was carried out on a Shimadzu GC-6A gas liquid chromatograph coupled to an FID detector, using a glass column (2 m × 3 mm I.D.) packed with 1.5% OV-17 on Chromosorb W. Proton nuclear magnetic resonance spectra were recorded on a JEOL 100 NMR spectrometer at 100 MHz in deuteriochloroform. Deuterium nuclear magnetic resonance spectra were recorded by a JEOL PFT-100/EC-100 pulsed Fourier transform spectrometer operating at 15.28 MHz with proton-noise decoupling. ^1H and ^2H chemical shifts are expressed in parts per million downfield from internal tetramethylsilane ($\delta=0$). All samples of chloroform solution were contained in 10 mm o.d. sample tubes. Field-frequency control was performed by reference to the internal signal of C_6F_6 (a few drops were added in chloroform solution). Abbreviations used: s=singlet; d=doublet; m=multiplet; bs=broad singlet. Mass spectra (MS) were determined on a JEOL D-100 spectrometer at an ionizing voltage of 75 eV. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh). The plates (precoated TLC plates, Silica Gel 60F-254, Merck) were developed in benzene–acetone (7 : 3 v/v). The compound were visualized under UV light and/or by spraying with conc. H_2SO_4 and heating on an electric heater. The optical rotations were measured on a JASCO DIP-SL automatic polarimeter with a cell of 10 cm light path length, and circular dichroism (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.

(−)-Dehydrogriseofulvin (1a)——**1a** was prepared essentially by the method of Taub *et al.*[2c] Recrystallization from benzene gave colorless needles, mp 272—274°C (lit.[2c] 276—279°C), $[\alpha]_D^{19}$ −35.6° ($c=0.25$, acetone). MS *m/e*: 350 (M^+) (for ^{35}Cl-compound, base peak), 319 (M^+-31), 308, 197, 168, 140. ^1H NMR δ (ppm): 1.78 (3H, bs, 6′-CH_3), 3.63 (3H, s, 2′-OCH_3), 3.98 (3H, s, 4-OCH_3), 4.04 (3H, s, 6-OCH_3), 5.66 (1H, bs, 3′-H), 6.14 (1H, s, 5-H), 6.16 (1H, bs, 5′-H). The molecular ellipticity $[\theta]$ ($c=1.0$ mg/ml): $[\theta]_{370}$ −530, $[\theta]_{350}$ −1050, $[\theta]_{302}$ −21280, $[\theta]_{290}$ 0, $[\theta]_{282}$ +19250, $[\theta]_{263}$ 0, $[\theta]_{258}$ −2800, $[\theta]_{255}$ 0, $[\theta]_{246}$ +13650, $[\theta]_{242}$ 0, $[\theta]_{235}$ −25550, $[\theta]_{228}$ 0.

(+)-Epigriseofulvin (3a)——**3a** was prepared by the method of MacMillan.[7a] Recrystallization from chloroform and then from benzene afforded **3a** as colorless needles, mp 218—220°C (lit.,[7a] 214—216°C), $[\alpha]_D^{19}$ +100.7° ($c=0.15$, $CHCl_3$). MS *m/e*: 352 (M^+) (for ^{35}Cl-compound), 321 (M^+-31), 310, 215, 214, 138 (base peak), 69. ^1H NMR δ (ppm): 0.90 (3H, d, $J=6$ Hz, 6′-CH_3), 2.40—3.00 (3H, m, 5′α,5′β and 6′β-H), 3.62 (3H, s, 2′-OCH_3), 3.98 (3H, s, 4-OCH_3), 4.01 (3H, s, 6-OCH_3), 5.57 (1H, bs, 3′-H), 6.13 (1H, s, 5-H). The molecular ellipticity $[\theta]$ ($c=1.0$ mg/ml): $[\theta]_{352}$ 0, $[\theta]_{340}$ −10490, $[\theta]_{332}$ −9330, $[\theta]_{324}$ −11300, $[\theta]_{300}$ 0, $[\theta]_{281}$ +26010, $[\theta]_{244}$ 0, $[\theta]_{237}$ −31470, $[\theta]_{232}$ 0, $[\theta]_{230}$ +15730.

(+)-Dehydrogriseofulvin (4a)——A solution of epigrieofulvin–$CHCl_3$ complex (1.0 g) and selenium dioxide (1.0 g) in *tert*-butanol (40 ml) was refluxed for 48 h. The ratio (1: 6) of the starting material and the product in the reaction mixture was determined by gas liquid chromatography. The hot reaction mixture was filtered and Darco-G-60 (1.0 g) was added. The mixture was filtered again after 15 min. After removal of the solvent under reduced pressure and extraction of the residue with benzene, the benzene extract was directly applied to column chromatography over silica gel in benzene, and eluted with benzene–methylene chloride (1: 9). Crude dehydrogriseofulvin, freed from the starting material, was rechromatographed over silica gel and eluted with benzene–methylene chloride (3: 7). The eluate gave colorless needles of (+)-dehydrogriseofulvin (**4a**) which were recrystallized from benzene, mp 271—273°C, $[\alpha]_D^{19}$ +35.2° (c=0.26, acetone). *Anal.* Calcd for $C_{17}H_{15}ClO_6$: C, 58.21; H, 4.31. Found: C, 58.60; H, 4.28. MS and ^1H NMR were identical with those of the enantiomer, (−)-dehydrogriseofulvin (**1a**). The molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{370}$ +530, $[\theta]_{350}$ +1050, $[\theta]_{302}$ +20300, $[\theta]_{292}$ 0, $[\theta]_{282}$ −17500, $[\theta]_{263}$ 0, $[\theta]_{258}$ +2800, $[\theta]_{255}$ 0, $[\theta]_{246}$ −12080, $[\theta]_{242}$ 0, $[\theta]_{235}$ +24850, $[\theta]_{228}$ 0. The mixed (1: 1) mp of (+)- and (−)-dehydrogriseofulvin was 297—299°C (dec.) with a phase change at 272°C.

(−)-Griseofulvin (5)——A suspension of 5% palladium–charcoal catalyst (175 mg) in an ethyl acetate solution (75 ml) of (+)-dehydrogriseofulvin (**4a**) (350 mg, 1 mmol) was shaken under a stream of hydrogen at atmospheric pressure and at room temperature. The hydrogenation was stopped after 90 min. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue 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, 272 mg). The crude product in benzene was chromatographed on silica gel and eluted with two solvent mixtures. 1) Elution with benzene–methylene chloride (1: 9) and recrystallization from benzene gave colorless plates of (−)-griseofulvin, mp 226—227°C, $[\alpha]_D^{21}$ −338.2° (c=0.15, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 58.33; H, 4.19. MS m/e: 352 (M^+) (for ^{35}Cl-compound), 321 (M^+-31), 310, 215, 214, 138 (base peak), 69. ^1H NMR δ (ppm): 0.96 (3H, d, J=6 Hz, 6′-CH_3), 2.20—3.20 (3H, m, 5′α,5′β and 6′α-H), 3.61 (3H, s, 2′-OCH_3), 3.97 (3H, s, 4-OCH_3), 4.02 (3H, s, 6-OCH_3), 5.53 (1H, bs, 3′-H), 6.12 (1H, s, 5-H). The molecular ellipticity $[\theta]$ (c=1.0 mg/ml): $[\theta]_{352}$ 0, $[\theta]_{323}$ −20240, $[\theta]_{292}$ −32980, $[\theta]_{268}$ −4440, $[\theta]_{237}$ −116440. The mixed (1: 1) mp of the (+)- and (−)-griseofulvin was 230—231°C with a phase change at 220°C. 2) Elution with benzene–methylene chloride (3: 7) and recrystallization from ethanol gave (+)-dihydrogriseofulvin as colorless needles, mp 204—207°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. MS m/e: 354 (M^+) (for ^{35}Cl-compound), 322, 255 (base peak). ^1H NMR δ (ppm): 0.92 (3H, d, J=6 Hz, 6′-CH_3), 1.60—3.20 (6H, m, aliphatic H), 3.27 (3H, s, 2′-OCH_3), 3.96 (3H, s, 4-OCH_3), 4.01 (3H, s, 6-OCH_3), 6.10 (1H, s, 5-H). 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 94 mg as a pale yellow oil. Recrystallization from benzene afforded griseophenone A as pale yellow needles, mp 217—218°C, undepressed on admixture with an authentic sample.[2c]

4-Methoxy-deuterated (+)-Griseofulvin——A methanol-d_1 (CH_3OD) solution of 4-demethylgriseofulvin (3.0g) was treated with a diethyl ether solution of partially deuterated diazomethane–diethyl ether complex (a solution of 50% KOH in D_2O was added to a suspension of nitrosomethylurea in diethyl ether) and the mixture was allowed to stand at 0°C for 30 min. The reaction mixture was concentrated *in vacuo*, and the crude product was extracted with chloroform (250 ml × 3). The chloroform solution was washed with 2 N sodium carbonate and water, dried (Na_2SO_4) and concentrated *in vacuo* to give a yellow oil (2.5 g). The benzene solution was subjected to column chromatography over silica gel in benzene. Elution with benzene–methylene chloride (3: 7) and recrystallization of the product from benzene gave the 4-methoxy-deuterated derivative of (+)-griseofulvin as colorless plates, mp 222—223°C, $[\alpha]_D^{26}$ +344.4° (c=0.18, $CHCl_3$); MS: $^2H_0$ 46.6, $^2H_1$ 38.9, $^2H_2$ 13.2, $^2H_3$ 1.3% (0.69 ^2H/molecule); ^1H NMR was identical with that of **2**, except for some decrease of the signal intensity at 3.97 ppm.

(±)-Dehydrogriseofulvin——A mixture of (+)-griseofulvin (2 g) and DDQ (2.6 g) in dioxane (50 ml) was refluxed for 48 h. After usual work-up, the neutral product (1 g) was dissolved in dioxane (20 ml) and allowed to react with DDQ (2.2 g) for 19 h. The neutral products (550 mg) were chromatographed on silica gel and eluted with benzene–methylene chloride (3: 7). Recrystallization of the product from benzene gave (±)-dehydrogriseofulvin as a colorless powder, mp 290—293°C (dec.), $[\alpha]_D^{19}$ 0° (c=0.24, acetone). *Anal.* Calcd for $C_{17}H_{15}ClO_6$: C, 58.21; H, 4.31. Found: C, 58.59; H, 4.23. ^1H NMR and MS; identical with those of (−)- and (+)-dehydrogriseofulvin.

(+)-[5′-^{2}H]Dehydrogriseofulvin (4c)——A solution of (+)-epigriseofulvin (3.0 g) in chloroform (210 ml) was mixed with neutral alumina (100 g) which had been activated with deuterium oxide (99.8% ^2H from Prochem) to activity II. The mixture was shaken at room temperature for 11 days, then the alumina was filtered off and washed with chloroform. Removal of the combined solvent *in vacuo* gave a white powder. Chromatography on silica gel and crystallization from benzene gave (+)-[5′α,5′β-^{2}H]epigriseofulvin as needles, mp 225—226°C, $[\alpha]_D^{19}$ +105.0° (c=0.16, $CHCl_3$). MS: $^2H_0$ 33.3%, $^2H_1$ 48.7%, $^2H_2$ 18.0% (0.85 ^2H/molecule). ^2H NMR δ (ppm): 2.6 (broad signal). *Anal.* Calcd for $C_{17}H_{18}ClO_6$: C, 57.71; H, 5.13. Found: C, 56.52; H, 4.64. Selenium dioxide (1.0 g) was added to a solution of (+)-[5′α,5′β-^{2}H]epigriseofulvin (0.82 g)

in *tert*-butanol (40 ml). The mixture was refluxed for 40 h. Chromatography of the neutral products on silica gel and recrystallization from benzene gave (+)-[5′-^{2}H]dehydrogriseofulvin as needles, mp 271—272°C, $[\alpha]_D^{24}$ +33.8° (c=0.24, acetone). MS: $^2H_0$ 56.6%, $^2H_1$ 43.4% (0.43 ^{2}H/molecule). ^{2}H NMR δ (ppm): 6.3 (broad signal). CD: identical with that of (+)-dehydrogriseofulvin. *Anal.* Calcd for $C_{17}H_{16}ClO_6$: C, 58.04; H, 4.59. Found: C, 57.97; H, 4.36.

Griseophenone A (6) and Diphenylether-carboxylic Acid Derivative (7)——6 (mp 217—218°C, lit.,[2c] 210—212°C) and 7 (mp 226—227°C, lit.,[10] 224—225°C) were prepared according to the methods of Taub *et al.*[2c] and Kyburz *et al.*,[10] respectively.

Microbial Transformation of (+)-Dehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——1) Spores of *Streptomyces cinereocrocatus* NRRL 3443 were inoculated[3] into 12 flaks each containing 20 ml of medium consisting of 3% cornstarch, 2% molasses, 4% cornsteep liquor, 1% calcium carbonate and 0.25% yeast extract with pH adjusted to 6.8. After incubation for 72 h at 28°C on a rotary shaker (100 rpm), aliquots of 5 ml of inoculum were used to inculate 500 ml Sakaguchi flasks containing 100 ml of sterile medium, and 5 mg of (+)-dehydrogriseofulvin in 2 ml of acetone was added to each flask. Incubation was continued for 72 h at 28°C. The fermented mash was separated by centrifugation into the mycelia and the supernatant. They were repeatedly extracted with chloroform, washed with 5% Na_2CO_3 and concentrated under reduced pressure. Gas liquid chromatography of the residue showed that griseofulvin and dehydrogriseofulvin were obtained in 33 and 6% yields from the supernatant, respectively, and in 10 and 4% yields from the mycelia, respectively. Column chromatography of the products from the supernatant on silica gel and recrystallization from benzene gave griseofulvin and dehydrogriseofulvin. (+)-Griseofulvin: mp 228—229°C, $[\alpha]_D^{20}$ +336.9° (c=0.12, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 56.84; H, 4.96. MS m/e: 352 (M^+)(for ^{35}Cl-compound), 321 (M^+-31), 310, 215, 214, 138 (base peak), 69. ^{1}H NMR δ (ppm): 0.96 (3H, d, J=6 Hz, 6′-CH_3), 2.20—3.20 (3H, m, 5′α,5′β and 6′α-H), 3.61 (3H, s, 2′-OCH_3), 3.97 (3H, s, 4-OCH_3), 4.02 (3H, s, 6-OCH_3), 5.53 (1H, bs, 3′-H), 6.12 (1H, s, 5-H). The molecular ellipticity $[\theta]$ (c=1.1 mg/ml): $[\theta]_{352}$ 0, $[\theta]_{323}$ +22530, $[\theta]_{292}$ +38370, $[\theta]_{268}$ +5630, $[\theta]_{237}$ +139390. (+)-Dehydrogriseofulvin: mp 271—274°C, $[\alpha]_D^{19}$ +34.8° (c=0.23, acetone). *Anal.* Calcd for $C_{17}H_{15}ClO_6$: C, 58.21; H, 4.31. Found: C, 58.62; H, 4.26. MS m/e: 350 (M^+) (for ^{35}Cl-compound, base peak), 319 (M^+-31), 308, 197, 168, 140. ^{1}H NMR δ (ppm): 1.78 (3H, bs, 6′-CH_3), 3.63 (3H, s, 2′-OCH_3), 3.98 (3H, s, 4-OCH_3), 4.04 (3H, s, 6-OCH_3), 5.66 (1H, bs, 3′-H), 6.14 (1H, s, 5-H), 6.16 (1H, bs, 5′-H).

2) The above fermentation was stopped after 24 h incubation and the broth was treated as described above, affording griseofulvin and dehydrogriseofulvin in yields of 12 and 40%, respectively. (+)-Griseofulvin: mp 226—227°C, $[\alpha]_D^{19}$ +336.5° (c=0.09, $CHCl_3$). (+)-Dehydrogriseofulvin: mp 272—273°C, $[\alpha]_D^{19}$ +36.0° (c=0.15, acetone).

Microbial Transformation of (−)-Dehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——Fermentation and separation were essentially the same as described above except that (−)-DGF was used as the substrate. Yields and physical data of GF and DGF thus obtained are shown in Tables II and III. The products obtained by fermentation for 8 days after the administration of (−)-DGF were column chromatographed on silica gel to give (+)-GF and 5′α-hydroxygriseofulvin, mp 218—220°C (lit.,[3] 222—224°C). MS m/e: 368 (M^+) (for ^{35}Cl-compound). ^{1}H NMR δ (ppm): 1.11 (3H, d, J=6 Hz, 6′-CH_3), 2.6 (1H, m, 6′α-H), 3.67 (3H, s, 2′-OCH_3), 4.01 and 4.05 (3H×2, singlet each, 4- and 6-OCH_3), 4.72 (1H, d, J=12 Hz, 5′β-H), 5.06 (1H, s, 3′-H), 6.18 (1H, s, 5-H). The acetate of 5′α-hydroxygriseofulvin: mp 258—260°C (lit.,[3] 254—256°C). MS m/e: 410 (M^+) (for ^{35}Cl-compound).

Microbial Transformation of (±)-Dehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——Fermentation and separation were essentially the same as described above except that (±)-dehydrogriseofulvin was used as the substrate, giving (+)-griseofulvin, mp 227—229°C. MS and CD spectra: identical with those of the standard sample of (+)-griseofulvin.

Microbial Transformation of (+)-[5′-^{2}H]Dehydrogriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——Fermentation and separation were essentially the same as described above, except that (+)-[5′-^{2}H]-dehydrigriseofulvin (**4c**) (300 mg) was used as the substrate, giving (+)-[5′α-^{2}H]GF, mp 229—230°C, $[\alpha]_D^{18}$ +333.3° (c=0.17, $CHCl_3$). CD spectrum: identical with that of the standard sample of (+)-griseofulvin. MS m/e: 352 (M^+); $^2H_0$ 57.4%, $^2H_1$ 42.6% (0.43 ^{2}H/molecule). ^{2}H NMR δ (ppm): 2.49 (bs). *Anal.* Calcd for $C_{17}H_{18}ClO_6$: C, 57.71; H, 5.13. Found: C, 57.54; H, 4.75. Recovered deuterated dehydrogriseofulvin, mp 274—275°C. CD spectrum: identical with that of the standard sample of substrate. MS: $^2H_0$ 59.7, $^2H_1$ 40.3 (0.40 ^{2}H/molecule). *Anal.* Calcd for $C_{17}H_{16}ClO_6$: C, 58.04; H, 4.59. Found: C, 58.63; H, 4.53.

Microbial Transformations of (−)- and (+)-Dehydrogriseofulvin by *Streptomyces roseochromogenus*, *S. bikiniensis*, *S. griseinus*, *S. durhamencis*, *S. californicus*, *S. fimbriatus*, *S. cinereoruber*——Fermentation and separation were essentially the same as described in the case of *S. cinereocrocatus*. Yields and physical data of griseofulvin and dehydrogriseofulvin obtained by the microbial transformation are shown in Tables II and III. The yield is the sum of those in the supernatant and the mycelia in each experiment.

Fermentation of (+)-Epigriseofulvin by *Streptomyces cinereocrocatus* NRRL 3443——30 mg of (+)-epigriseofulvin (**3a**) was fermentated with *S. cinereocrocatus* under essentially the same conditions as described above. The yields of recovered **3a** were 85 and 15% in the supernatant and the mycelia, respectively, as determined by GLC, indicating complete recovery of the starting material. In a parallel experiment using

(−)-DGF as the substrate, (+)-griseofulvin was obtained in 60% yield from the supernatant.

Fermentation of Griseophenone A and Diphenyl Carboxylic Acid Derivative by *Streptomyces cinereocrocatus* NRRL 3443——Griseophenone A (**6**) or diphenyl carboxylic acid (**7**) (10 mg) was fermented as described above. No DGF or GF was detected as a product by GLC and TLC in either case.

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