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

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To elucidate the stereochemistry of microbial hydrogenation of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1** and **2**) by *Streptomyces cinereocrocatus* NRRL 3443, deuterated substrates (**1a**, **1b**, **2a**, and **2b**) were synthesized from (+)-griseofulvin (**9**) and subjected to microbial transformation. The 41.41 MHz ^2H nuclear magnetic resonance (NMR) and 400 MHz ^1H NMR studies clearly revealed that the microbial hydrogenations of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1** and **2**) proceed stereospecifically with an *anti*-addition of hydrogens at the 2′ and 3′ positions. Further, the microbial transformation of **1** to (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5**) indicates an isomerization by the microorganism of **1** to the enantiomer, (−)-2′-demethoxydehydrogriseofulvin (**2**), which is further hydrogenated to afford **5**.

Keywords——microbial transformation; ^2H NMR; enantiomer; *Streptomyces cinereocrocatus*; deuterated (+)-griseofulvin derivative; deuterated (−)-griseofulvin derivative; antibiotic; stereochemistry; *re* attack; *si* attack

In a preceding paper,[1] we reported studies on the microbial transformation of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1** and **2**) by *Streptomyces cinereocrocatus*, which demonstrated that the products formed from **1** were (+)-2′-demethoxygriseofulvin (**3**) and (−)- and (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**4** and **5**), whereas only **5** was obtained from **2** (Chart 1).

In this paper, therefore, the microbial transformations of the deuterated substrates (**1a**, **1b**, **2a**, and **2b**) were performed using *Streptomyces cinereocrocatus*, and the stereochemistry of the transformation products was elucidated from 400 MHz proton nuclear magnetic resonance (^1H NMR), 41.41 MHz ^2H NMR and circular dichroism (CD) spectral data.

Results and Discussion

Syntheses of Deuterated Substrates

(+)-[2′-^2H]- and [3′-^2H]-2′-demethoxydehydrogriseofulvin (**1a** and **1b**) and their enantiomers (**2a** and **2b**) were synthesized as follows (Chart 2). Compounds **1a** ($^2H_0$ 58.2%, $^2H_1$ 41.8%) and **1b** ($^2H_0$ 68.8%, $^2H_1$ 31.2%) were prepared from **3a** ($^2H_0$ 51.2%, $^2H_1$ 48.8%) and **3b** ($^2H_0$ 35.5%, $^2H_1$ 60.9%, $^2H_2$ 3.6%), respectively, as described previously.[1] The ^1H NMR of **1a** showed some decrease of the signal intensity at the olefinic 2′-H, and the 3′-H region appeared as a nearly singlet signal (δ 6.41 ppm) which was as expected for the 2′-deuterated compound. In the case of **1b**, the ^1H NMR exhibited some decrease of the signal intensity at the olefinic 3′-H, and the 2′-H region appeared as a nearly singlet signal (δ 6.55 ppm) which was as expected for the 3′-deuterated compound. On the other hand, **2a** ($^2H_0$ 39.6%, $^2H_1$ 60.4%) was prepared from **6** as described previously,[1] except for the use of deuterium in the catalytic hydrogenation of **6**. The ^1H NMR spectrum of **2a** was apparently identical with

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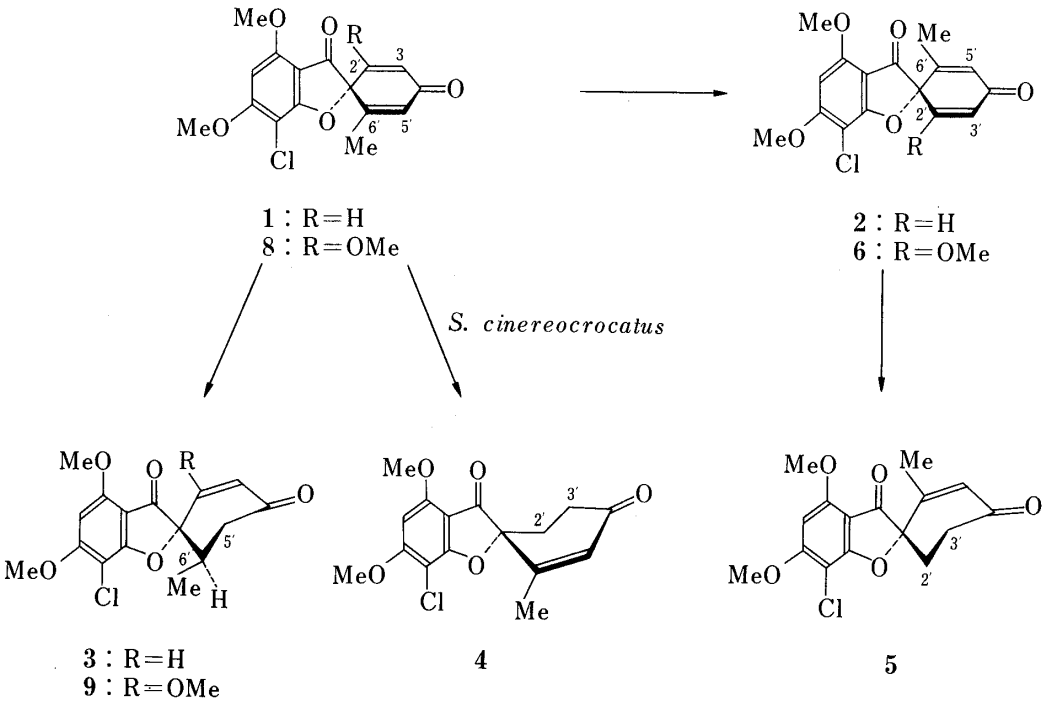
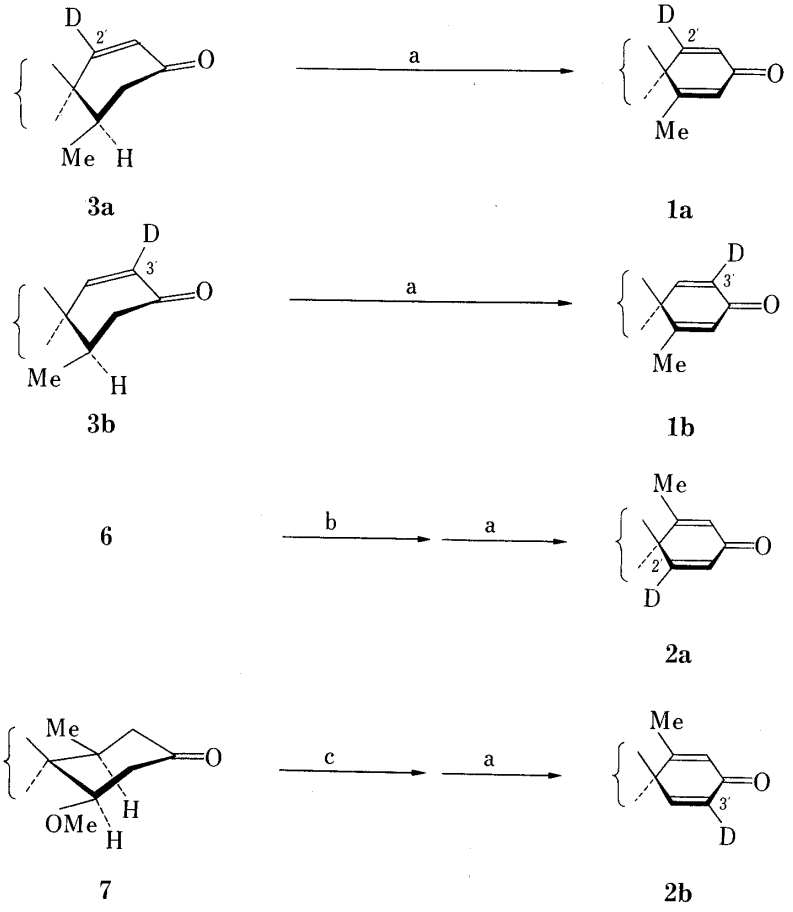


Chart 1



reagents: a, 1) $PyHBr_3/CHCl_3$, 2) LiCl, Li_2CO_3/DMF
b, 1) $^2H_2$/Pd-C/AcOEt, 2) H_2SO_4/EtOH
c, D_2SO_4/EtOD

Chart 2

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that of **1a**. Further, **2b** ($^2H_0$ 35.8%, $^2H_1$ 57.9%, $^2H_2$ 6.3%) was prepared from **7** as described previously,[1] except for the use of D_2SO_4–EtOD solution in the acid treatment of **7**. The ^1H NMR spectrum of **2b** was apparently identical with that of **1b** except for some decrease of the signal intensity at 5′-H.

Deuterated Standard Samples of (+)- and (−)-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvins for Microbial Transformation

The structural formulae of the deutrated standard samples (**4a**, **4b**, **4c**, and **4d**) are shown in Chart 3. Compounds **4a**, **4b**, and **4c** were prepared as described previously,[1] and the configurations of the deuteron(s) in each compound were determined on the basis of ^1H NMR data. Compound **4d** ($^2H_0$ 15.9%, $^2H_1$ 35.5%, $^2H_2$ 37.2%, $^2H_3$ 8.3%, $^2H_4$ 3.1%) was prepared by treatment of **4** with neutral alumina (Woelm, activity II D_2O) in $CHCl_3$ for 8 h. Parts of the 41.41 MHz ^2H NMR spectra of the four samples are shown in Fig. 1. The ^2H NMR of **4a** showed two signals at 2.46 and 2.55 ppm which were assignable to 2′α- and 3′α-^2H, respectively. The ^2H NMR of **4b** showed a signal at 2.46 ppm and that of **4c** showed a broad signal at 2.32 ppm, demonstrating that **4b** and **4c** are [2′α-^2H]- and [2′β-^2H]-**4**, respectively. The ^2H NMR of **4d** showed four signals at 1.80, 2.55, 2.93, and 6.09 ppm which were assignable to 6′-methyl, 3′α-, 3′β-, and 5′-^2H. This result indicates that 5′-H and 6′-methyl are also deuteron-substituted under the conditions of D_2O-activated neutral alumina treatment of **4**.

Stereochemistry of Microbial Transformation of (−)-2′-Demethoxydehydrogriseofulvin

The microbial transformations of (−)-[2′-^2H]- and (−)-[3′-^2H]-2′-demethoxydehydrogriseofulvins (**2a** and **2b**) by *S. cinereocrocatus* were performed under the same conditions as described in the previous paper.[1] After a 12 h fermentation of **2a**, deuterated (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5a**, $^2H_0$ 38.2%, $^2H_1$ 51.9%) was isolated as the transformation product from the broth. Since the ^2H NMR signal of **5a** appeared at the same chemical shift as that of the 2′β-^2H signal of **4c**, the configuration of the deuteron was unequivocally assignable as 2′β (Chart 4). On the other hand, after the 12 h fermentation of **2b**, deuterated (+)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5b**, $^2H_0$ 34.1%, $^2H_1$ 51.6%, $^2H_2$ 14.3%) was isolated from the broth. Since the ^2H NMR signal of **5b** appeared at the same chemical shift as the 3′α-^2H signal of the [2′α,3′α-^2H]- and [3′α-, 3′β-^2H]-compounds (**4a** and **4d**), the configuration of the deuteron was also unequivocally assignable as 3′α (Fig. 2). The above ^2H NMR results clearly demonstrate that the microbial hy-

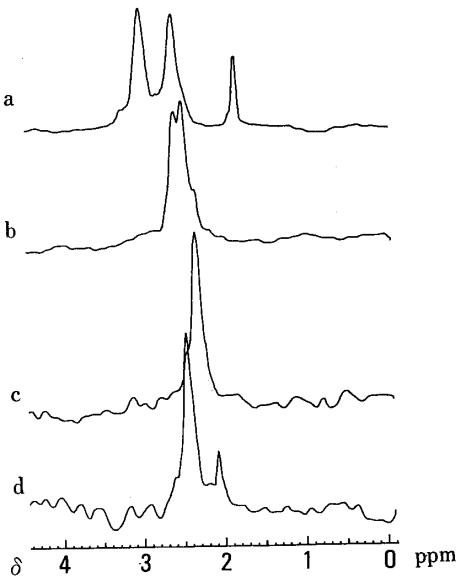
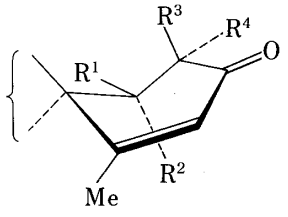


Fig. 1. Parts of the 41.41 MHz ^2H NMR Spectra of Partially Deuterated Compounds (**4a**, **4b**, **4c**, and **4d**)

a, **4d**; b, **4a**; c, **4c**; d, **4b**.



4a: $R^2=R^4=^2H$, $R^1=R^3=H$
4b: $R^2=^2H$, $R^1=R^3=R^4=H$
4c: $R^1=^2H$, $R^2=R^3=R^4=H$
4d: $R^3=R^4=^2H$, $R^1=R^2=H$

Chart 3

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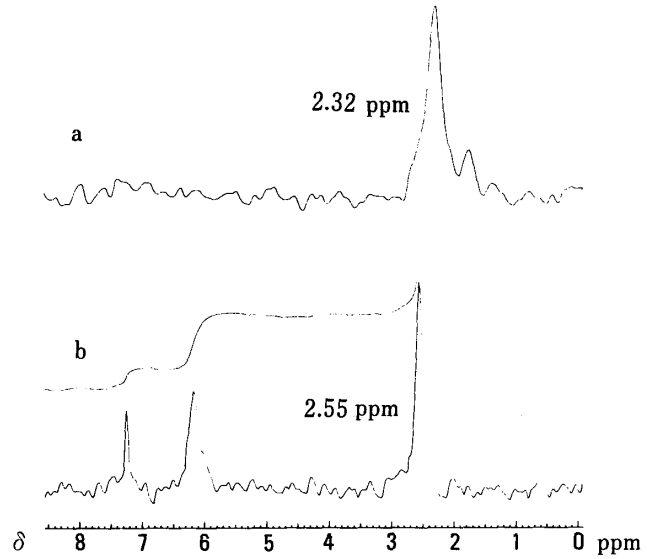

Fig. 2. The 41.41 MHz ^2H NMR Spectra of Microbial Transformation Products
a, **5a**; b, **5b**.

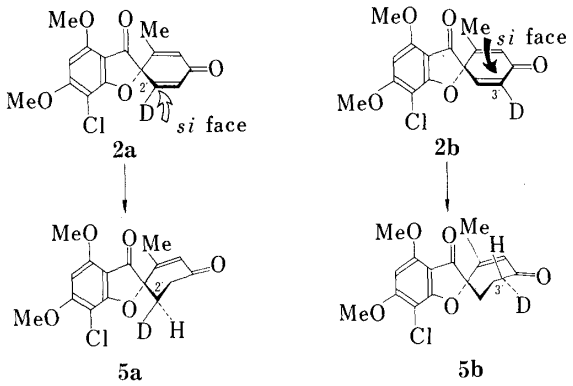

Chart 4

drogenation of (−)-2′-demethoxydehydrogriseofulvin (**2**) proceeds with *anti*-addition of hydrogens *via si* attacks at the 2′ and 3′ positions (Chart 4).

Stereochemistry of Microbial Transformation of (+)-2′-Demethoxydehydrogriseofulvin

The microbial transformations of (+)-[2′-^2H]- and [3′-^2H]-2′-demethoxydehydrogriseofulvins (**1a** and **1b**) by *S. cinereocrocatus* were performed under the same conditions as described above for the (−)-isomers. After 12 h, the transformation product ($^2H_0$ 59.2%, $^2H_1$ 40.8%) from **1a** was isolated from the broth, and shown to be composed of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5c** and **4e**) in a relative ratio of 89 : 11 by comparisons of its CD spectrum with those of standard (+)- and (−)-compounds (**5** and **4**).[1] Since the 400 MHz ^1H NMR of the mixture of **5c** and **4e** showed almost the same pattern as that of the 2′β-deuterated standard sample (**4c**), the configuration of the deuteron of the microbial transformation products was unequivocally assignable as 2′β (Chart 5). Next, in the microbial transformation of **1b**, the products ($^2H_0$ 73.9%, $^2H_1$ 26.1%) consisted of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5d** and **4f**), in a relative ratio of 91 : 9, based on comparisons of its CD spectrum with those of standard (+)- and (−)-compounds, after 12 h incubation. Since the 400 MHz ^1H NMR spectrum was identical with that of **4** except for some decrease of the signal intensity at the 3′α-H region, the configuration of the deuteron was unequivocally assignable as 3′α (Chart 5). The results

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mentioned above clearly indicate that the microbial hydrogenations of **1a** and **1b** to **4e** and **4f** proceed with *anti*-addition of hydrogens *via re* attacks at the 2′ and 3′ positions. That is, in the microbial hydrogenations of **1a** and **1b**, the compounds were first converted to their corresponding enantiomers (**2a** and **2b**), followed by *anti*-addition of hydrogens *via si* attacks at the 2′ and 3′ positions to give **5c** and **5d**.

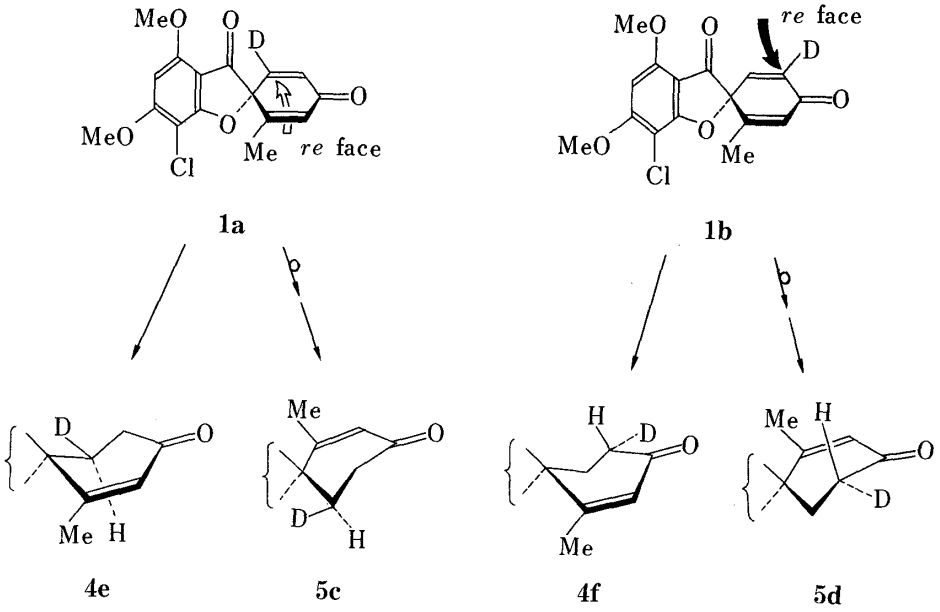

Chart 5

In conclusion, the 41.41 MHz ^{2}H NMR and 400 MHz ^{1}H NMR studies clearly showed that the microbial hydrogenations of (+)- and (−)-2′-demethoxydehydrogriseofulvin (**1** and **2**) proceed stereospecifically[2] with *anti*-addition of hydrogens at the 2′,3′ positions. Comparison of the above results with those[2,3] for (−)- and (+)-dehydrogriseofulvin (**8** and **6**) indicates that the microbial hydrogenation of dehydrogriseofulvin analogs by *S. cinereocrocatus* proceeds with the same stereochemistry in a *trans* diaxial manner regardless of the 2′-substituent.

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 chromatograph with a flame ionization detector and nitrogen as the carrier gas. A glass column (2 m × 3 mm i.d.) of 1.5% OV-17 on Chromosorb W was used. Proton nuclear magnetic resonance spectra were obtained on JEOL JNM-MH-100 and JEOL FX-400 NMR machines. All proton nuclear magnetic resonance spectra were recorded in deuteriochloroform and signals are reported as parts per million downfield from Me_4Si ($\delta = 0$). Abbreviations used: s = singlet, d = doublet, t = triplet, br = broad, m = multiplet, and dd = double doublet. ^{2}H NMR spectra were recorded with a JEOL JNM-FX 270 spectrometer operated at 41.41 MHz in the proton-decoupled Fourier transform mode; no lock system was used with this spectrometer. The deuterium resonance of $CDCl_3$ ($\delta = 7.25$) served as a secondary chemical shift reference in ^{2}H spectra. All samples dissolved in chloroform were contained in 10 mm o.d. sample tubes. Spectra were usually taken under the following conditions: spectral width 500 Hz, 2K data points, 90° pulse (20 μs) and repetition time of 1 s. Mass spectra (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 (4 accumulations) from 380 to 230 nm. Column chromatography was performed with Kanto Kagaku gel (100 mesh). Thin layer chromatography was carried out by using 5 × 20 cm plates with 0.25 mm thickness of Merck silica gel 60F-254, developed with benzene–acetone (7 : 3 v/v), and spots were visualized by exposure to ultraviolet (UV) light and/or by heating the plate on an electric heater after spraying it with conc. H_2SO_4.

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(+)-[2′-^{2}H]-2′-Demethoxydehydrogriseofulvin (1a)——A mixture of **3a** (2.5 g) ($^2H_0$ 51.2%, $^2H_1$ 48.8%)[1] and pyridinium hydrobromide perbromide ($PyHBr_3$) (3 g) in chloroform (65 ml) was refluxed for 2 h. The reaction mixture was poured into a large volume of ice and water, and extracted with chloroform (500 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 3.1 g). The ratio (40 : 60) of the two products (3′-bromo and 5′α-bromo derivatives) was determined by gas-liquid chromatography. A solution of the mixture of bromo derivatives (3.1 g), LiCl (330 mg), Li_2CO_3 (570 mg) and pyridine (25 ml) in *N*-dimethylformamide (DMF) (200 ml) was heated at 100 °C for 18 h, then the reaction mixture was poured into ice and water, and extracted with chloroform (150 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 1.8 g). The residue was chromatographed on silica gel (160 g) and eluted with benzene–methylene chloride (50 : 50). The eluate gave colorless needles of (+)-[2′-^{2}H]-2′-demethoxydehydrogriseofulvin (**1a**), which were recrystallized from benzene, mp 254—257 °C. MS: $^2H_0$ 58.2%, $^2H_1$ 41.8%. ^{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 and s, 3′-H), 6.55 (0.6H, d, J=10.0 Hz, 2′-H). Molecular ellipticity [θ] (c=0.9 mg/ml): $[\theta]_{370}$ −530, $[\theta]_{343}$ −3820, $[\theta]_{327}$ 0, $[\theta]_{300}$ +9590, $[\theta]_{284}$ 0, $[\theta]_{270}$ −5980, $[\theta]_{263}$ 0, $[\theta]_{255}$ +7180, $[\theta]_{248}$ +5170, $[\theta]_{238}$ +49720.

(+)-[3′-^{2}H]-2′-Demethoxydehydrogriseofulvin (1b)——A mixture of **3b** (740 mg) ($^2H_0$ 35.5%, $^2H_1$ 60.9%)[1] and $PyHBr_3$ (870 mg) in chloroform (18 ml) was refluxed for 2 h. The reaction mixture was poured into a large volume of ice and water, and extracted with chloroform (150 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 880 mg). The ratio (30 : 70) of the two products (3′-bromo and 5′α-bromo derivatives) was determined by gas liquid chromatography. A solution of the mixture of bromo derivatives (880 mg), LiCl (94 mg), Li_2CO_3 (160 mg) and pyridine (5 ml) in DMF (70 ml) was heated at 100 °C for 17 h. After usual treatment of the reaction mixture, the residue (740 mg) was chromatographed on silica gel (70 g) and eluted with benzene–methylene chloride (50 : 50). The eluate gave colorless needles of (+)-[3′-^{2}H]-2′-demethoxydehydrogriseofulvin (**1b**), which were recrystallized from benzene, mp 258—259 °C. MS: $^2H_0$ 68.8%, $^2H_1$ 31.2%.^{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 (0.6H, d, J=10.0 Hz, 3′-H), 6.55 (1H, d, J=10.0 Hz, and s, 2′-H). Molecular ellipticity [θ] (c=1.0 mg/ml): $[\theta]_{370}$ −540, $[\theta]_{343}$ −3890, $[\theta]_{327}$ 0, $[\theta]_{300}$ +11190, $[\theta]_{284}$ 0, $[\theta]_{270}$ −6250, $[\theta]_{263}$ 0, $[\theta]_{255}$ +7510, $[\theta]_{248}$ +5400, $[\theta]_{238}$ +51710.

(−)-[2′-^{2}H]-2′-Demethoxydehydrogriseofulvin (2a)——A suspension of 5% palladium–charcoal catalyst (600 mg) in an ethyl acetate solution (200 ml) of **6** (1 g)[1] was shaken under a stream of deuterium (purity 98%, Nissan Shoji Co., Ltd.) at atmospheric pressure and at room temperature. Reduction was stopped after 2 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue (980 mg) was dissolved in chloroform and washed with 2 N sodium hydroxide. The neutral product (640 mg) thus obtained was dissolved in benzene, and the solution was subjected to column chromatography on silica gel (60 g) in benzene. Elution with benzene–methylene chloride (60 : 40) (yield, 480 mg) and recrystallization of the product from benzene gave deuterated (+)-dihydrogriseofulvin as colorless needles, mp 218—219 °C. MS: $^2H_0$ 1.3%, $^2H_1$ 4.7%, $^2H_2$ 18.5%, $^2H_3$ 44.3%, $^2H_4$ 31.1%. The yellow alkaline extract, after acidification with 10% HCl, was extracted with chloroform. 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. Recrystallization from benzene afforded griseophenone A as pale yellow needles, mp 218—219 °C. MS: $^2H_0$ 88.0%, $^2H_1$ 6.5%, $^2H_2$ 5.5%. The deuterated (+)-dihydrogriseofulvin (450 mg) was heated under reflux with 2 N H_2SO_4 (55 ml) and ethanol (40 ml) for 6 h. After neutralization with 5 N Na_2CO_3, the mixture was concentrated *in vacuo*. The solid (420 mg) obtained by filtration was chromatographed in benzene on silica gel (50 g) and eluted with benzene–methylene chloride (50 : 50). Recrystallization from methanol gave deuterated (−)-2′-demethoxygriseofulvin as colorless needles, mp 182—183 °C. MS: $^2H_0$ 4.0%, $^2H_1$ 33.2%, $^2H_2$ 53.6%, $^2H_3$ 9.2%. A mixture of deuterated (−)-2′-demethoxygriseofulvin (420 mg) and $PyHBr_3$ (500 mg) in chloroform (10 ml) was refluxed for 2 h. The reaction mixture was poured into a large volume of ice and water, and extracted with chloroform (100 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 560 mg). A solution of the mixture of bromo derivatives (3′-bromo : 5′α-bromo, 40 : 60) (560 mg), LiCl (60 mg), Li_2CO_3 (104 mg) and pyridine (2 ml) in DMF (44 ml) was heated at 100 °C for 23 h. The reaction mixture was poured into ice and water, and extracted with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 372 mg). The residue was chromatographed on silica gel (40 g) and eluted with benzene–methylene chloride (50 : 50). The eluate gave colorless needles of (−)-[2′-^{2}H]-2′-demethoxydehydrogriseofulvin (**2a**) which were recrystallized from benzene, mp 256—258 °C. MS: $^2H_0$ 39.6%, $^2H_1$ 60.4%. ^{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, and s, 3′-H), 6.55 (0.3H, d, J=10.0 Hz, 2′-H). Molecular ellipticity [θ] (c=1.1 mg/ml): $[\theta]_{370}$ +550, $[\theta]_{343}$ +3860, $[\theta]_{327}$ 0, $[\theta]_{300}$ −10970, $[\theta]_{284}$ 0, $[\theta]_{270}$ +6280, $[\theta]_{263}$ 0, $[\theta]_{255}$ −7320, $[\theta]_{248}$ −5380, $[\theta]_{238}$ −51850.

(−)-[3′-^{2}H]-2′-Demethoxydehydrogriseofulvin (2b)——**7** (470 mg) was heated under reflux with 2 N D_2SO_4 (56 ml) and EtOD (42 ml) (purity 98%, Merck Ltd.) for 6 h. After neutralization with 5 N Na_2CO_3, the mixture was concentrated *in vacuo*. The solid (432 mg) obtained by filtration was chromatographed on silica gel (50 g) and eluted with benzene–methylene chloride (50 : 50). Recrystallization from methanol gave deuterated (−)-2′-demethoxy-

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griseofulvin as colorless needles, mp 182—183 °C. MS: $^2H_0$ 0%, $^2H_1$ 0.7%, $^2H_2$ 2.8%, $^2H_3$ 40.3%, $^2H_4$ 56.2%. A mixture of deuterated (−)-2′-demethoxygriseofulvin (420 mg) and $PyHBr_3$ (500 mg) in chloroform (10 ml) was refluxed for 2 h. Usual work-up of the reaction mixture gave a mixture of bromo derivatives (yield, 530 mg). The ratio (35:65) of the two products (3′-bromo and 5′α-bromo derivatives) was determined by gas liquid chromatography. A solution of the mixture of bromo derivatives (530 mg), LiCl (56 mg), Li_2CO_3 (98 mg) and pyridine (2 ml) in DMF (42 ml) was heated at 100 °C for 20 h. After usual treatment of the reaction mixture, the residue (310 mg) was chromatographed on silica gel (40 g) and eluted with benzene–methylene chloride (50:50). The eluate gave colorless needles of (−)-[3′-^{2}H]-2′-demethoxydehydrogriseofulvin (**2b**) which were recrystallized from benzene, mp 257—259 °C. MS: $^2H_0$ 35.8%, $^2H_1$ 57.9%, $^2H_2$ 6.3%. ^{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 (0.7H, s, 5′-H), 6.41 (0.5H, d, J=10.0 Hz, 3′-H), 6.55 (1H, d, J=10.0 Hz and s, 2′-H). Molecular ellipticity [θ] (c=1.0 mg/ml): $[\theta]_{370}$ +510, $[\theta]_{343}$ +3710, $[\theta]_{327}$ 0, $[\theta]_{300}$ −10450, $[\theta]_{284}$ 0, $[\theta]_{270}$ +6070, $[\theta]_{263}$ 0, $[\theta]_{255}$ −7410, $[\theta]_{248}$ −5390, $[\theta]_{238}$ −50550.

[2′α,3′α-^{2}H]-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (4a)——Synthesis and physical data except for ^{2}H NMR data were described in a previous paper.[1] ^{2}H NMR δ (ppm): 2.46 (br signal, 2′α-D), 2.55 (br signal, 3′α-D).

[2′α-^{2}H]-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (4b)——Synthesis and physical data except for ^{2}H NMR data were described in a previous paper.[1] ^{2}H NMR δ (ppm): 2.46 (br signal, 2′α-D).

[2′β-^{2}H]-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (4c)——Synthesis and physical data except for ^{2}H NMR data were described in a previous paper.[1] ^{2}H NMR δ (ppm): 2.32 (br signal, 2′β-D).

[3′α,3′β-^{2}H]-2′-Demethoxy-2′,3′-dihydrodehydrogriseofulvin (4d)——A suspension of neutral alumina (Woelm, activity II, D_2O) in a chloroform (2.8 ml) solution of **4** (43 mg) was stirred at room temperature for 8 h. The alumina was removed by filtration and the filtrate was concentrated *in vacuo*. The residue (41.6 mg) was subjected to column chromatography on silica gel (20 g) in benzene. Elution with benzene–methylene chloride (40:60) and recrystallization of the product from methanol gave [3′α,3′β-^{2}H]-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**4d**) as colorless needles, mp 211—212 °C. MS: $^2H_0$ 15.9%, $^2H_1$ 35.5%, $^2H_2$ 37.2%, $^2H_3$ 8.3%, $^2H_4$ 3.1%. ^{1}H NMR δ (ppm): 1.79 (3H, s, 6′-Me), 2.32 (1H, dd, J=15.2 and 5.0 Hz, 2′β-H), 2.46 (1H, m, 2′α-H), 2.58 (0.4H, m, 3′α-H), 2.95 (0.4H, m, 3′β-H), 3.98 (3H, s, 4-OMe), 4.03 (3H, s, 6-OMe), 6.10 (0.7H, br s, 5′-H), 6.15 (1H, s, 5-H). ^{2}H NMR δ (ppm): 1.80 (br signal, 6′-Me) 2.55 (br signal, 3′α-D), 2.93 (br signal, 3′β-D), 6.09 (br signal, 5′-D).

Microbial Transformation of (+)-[2′-^{2}H]-2′-Demethoxydehydrogriseofulvin (1a) by *Streptomyces cinereocrocatus* NRRL 3443——All of the experiments were essentially the same as described in the previous paper,[1] except that (+)-[2′-^{2}H]-2′-demethoxydehydrogriseofulvin (**1a**) was used as the substrate. Silica gel column chromatography of the residue obtained from the 12 h incubation supernatant and recrystallization of the product from methanol gave an 89:11 mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5c** and **4e**) and (+)-2′-demethoxygriseofulvin. A mixture of (+)- and (−)-[2′β-^{2}H]-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5c** and **4e**): mp 218—220 °C. MS: $^2H_0$ 59.2%, $^2H_1$ 40.8%. The ^{1}H NMR spectrum was identical with that of **4**, except for some decrease of the signal intensity at 2.32 ppm and some difference in the 2.46, 2.58, and 2.98 ppm regions. The molecular ellipticity [θ] (c=0.9 mg/ml): $[\theta]_{370}$ +108, $[\theta]_{336}$ +16770, $[\theta]_{333}$ +17300, $[\theta]_{322}$ +24830, $[\theta]_{311}$ +20060, $[\theta]_{291}$ 0, $[\theta]_{264}$ −14010, $[\theta]_{242}$ 0, $[\theta]_{234}$ +92580. (+)-[2′-^{2}H]-2′-Demethoxygriseofulvin: mp 221—222 °C. MS: $^2H_0$ 61.0%, $^2H_1$ 39.0%.

Microbial Transformation of (+)-[3′-^{2}H]-2′-Demethoxydehydrogriseofulvin (1b) by *Streptomyces cinereocrocatus* NRRL 3443——All of the procedures were the same as described above, except that (+)-[3′-^{2}H]-2′-demethoxydehydrogriseofulvin (**1b**) was used as the substrate. Silica gel column chromatography of the residue from the 12 h incubation supernatant and recrystallization of the product from methanol gave a 91:9 mixture of (+)- and (−)-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5d** and **4f**) and (+)-2′-demethoxygriseofulvin. A mixture of (+)- and (−)-[3′α-^{2}H]-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5d** and **4f**): mp 218—220 °C. MS: $^2H_0$ 73.9%, $^2H_1$ 26.1%. The ^{1}H NMR spectrum was identical with that of **4**, except for some decrease of the signal intensity at 2.58 ppm and some difference in the 2.32, 2.46 and 2.98 ppm regions. The molecular ellipticity [θ] (c=0.9 mg/ml): $[\theta]_{370}$ +130, $[\theta]_{336}$ +17300, $[\theta]_{333}$ +17700, $[\theta]_{322}$ +25360, $[\theta]_{311}$ +20590, $[\theta]_{291}$ 0, $[\theta]_{246}$ −14410, $[\theta]_{242}$ 0, $[\theta]_{234}$ +95420. (+)-[2′-^{2}H]-2′-Demethoxygriseofulvin: mp 221—222 °C. MS: $^2H_0$ 71.2%, $^2H_1$ 28.8%.

Microbial Transformation of (−)-[2′-^{2}H]-2′-Demethoxydehydrogriseofulvin (2a) by *Streptomyces cinereocrocatus* NRRL 3443——All of the procedures were the same as described above, except that (−)-[2′-^{2}H]-2′-demethoxydehydrogriseofulvin (**2a**) was used as the substrate. Silica gel column chromatography of the residue from the 12 h incubation supernatant and recrystallization of the product from methanol gave (+)-[2′β-^{2}H]-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5d**), mp 218—220 °C. MS: $^2H_0$ 48.2%, $^2H_1$ 51.9%. ^{2}H NMR δ (ppm): 2.32 (br signal, 2′β-D). The ^{1}H NMR spectrum was identical with that of **5**, except for some decrease of the signal intensity at 2.32 ppm and some difference in the 2.46, 2.58, and 2.98 ppm regions. The CD spectrum was identical with that of **5**.[1]

Microbial Transformation of (−)-[3′-^{2}H]-2′-Demethoxydehydrogriseofulvin (2b) by *Streptomyces cinereocrocatus* NRRL 3443——All of the procedures were the same as described above, except that (−)-[3′-^{2}H]-2′-demethoxydehydrogriseofulvin (**2b**) was used as the substrate. Silica gel column chromatography of the residue from the 12 h incubation supernatant and recrystallization of the product from methanol gave (+)-[3′α-^{2}H]-2′-demethoxy-2′,3′-dihydrodehydrogriseofulvin (**5b**), mp 220—221 °C. MS: $^2H_0$ 34.1%, $^2H_1$ 51.6%, $^2H_2$ 14.3%. ^{2}H NMR δ (ppm):

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2.55 (br signal, 3′α-D), 6.17 (br signal, 5′-D). The ^{1}H NMR spectrum was identical with that of **6**, except for some decrease of the signal intensities at 2.58 and 6.15 ppm, and some difference in the 2.32, 2.46 and 2.98 ppm regions. The CD spectrum was identical with that of **5**.[1)]

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