Regio- and Stereoselective Hydrogenation of 2′-Demethoxy-2′-methyldehydrogriseofulvin, a Symmetrical Substrate, to (+)-2′-Demethoxy-2′-methylgriseofulvin with a Cell-Free System of *Streptomyces cinereocrocatus*

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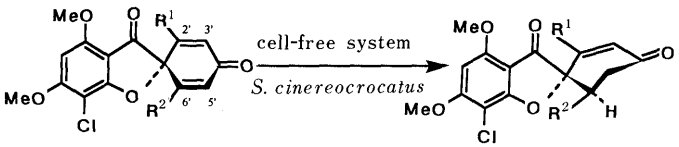
Enzymatic hydrogenation of 2′-demethoxy-2′-methyldehydrogriseofulvin (5) with a cell-free system of *Streptomyces cinereocrocatus* afforded (+)-2′-demethoxy-2′-methylgriseofulvin (6). The structure of 6 was determined on the basis of comparisons of the proton nuclear magnetic resonance spectrum, mass spectrum, and circular dichroism with those of a standard specimen which was synthesized chemically. The results demonstrated that when the 2′-position of (−)-dehydrogriseofulvin was substituted with a methyl group, its hydrogenation with the cell-free system occurred stereoselectively at the 5′,6′-position.

Keywords cell-free system; *Streptomyces cinereocrocatus* NRRL 3443; stereochemistry; 2′-demethoxy-2′-methyldehydrogriseofulvin; 2′-demethoxy-2′-methylgriseofulvin; methylation of α,β-unsaturated compound; enzymatic hydrogenation

We recently demonstrated the stereospecific microbial transformation of (−)-dehydrogriseofulvin (**1**) derivatives by *Streptomyces cinereocrocatus* NRRL 3443. In particular, the stereospecific microbial transformation of both (−)-dehydrogriseofulvin (**1**) and (+)-dehydrogriseofulvin (**2**) to (+)-griseofulvin (**3**) has been investigated (Chart 1).[1] However, the microbial transformation of (+)-2′-demethoxydehydrogriseofulvin (**4**) non-regiospecifically afforded two reduced products.[2] This paper describes the enzymatic hydrogenation of 2′-demethoxy-2′-methyldehydrogriseofulvin (**5**), which is a symmetrical substrate, with a cell-free system of *Streptomyces cinereocrocatus* NRRL 3443.



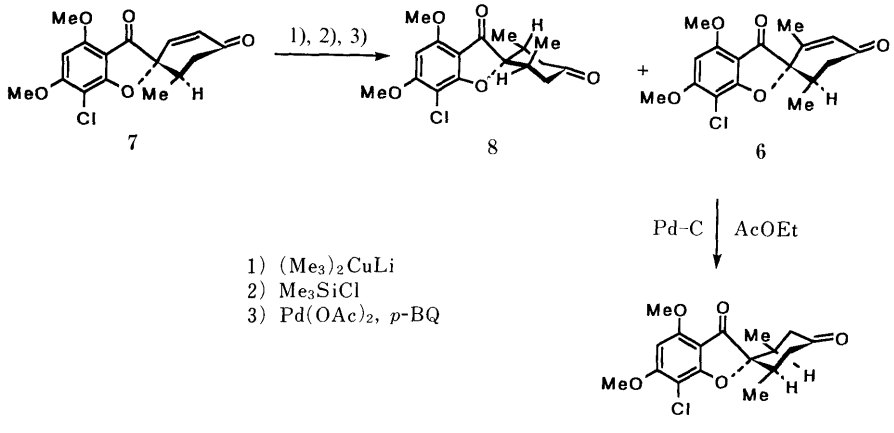
1 : R^1=OMe, R^2=Me
2 : R^1=Me, R^2=OMe
4 : R^1=H, R^2=Me
5 : R^1=Me, R^2=Me
13 : R^1=OPr, R^2=Me
14 : R^1=Me, R^2=OPr
15 : R^1=Me, R^2=H

3 : R^1=OMe, R^2=Me
6 : R^1=Me, R^2=Me
7 : R^1=H, R^2=Me
16 : R^1=OPr, R^2=Me
17 : R^1=Me, R^2=H

Chart 1

Results and Discussion

Synthesis of 2′-Demethoxy-2′-methylgriseofulvin Synthesis of α,β-unsaturated compounds by palladium(II)-catalyzed dehydrosilylation of silyl enol ethers has been reported.[3] Based on a combination of this dehydrosilylation with the preparation of silyl enol ethers from enolates, a procedure for the synthesis of (+)-2′-demethoxy-2′-methylgriseofulvin (**6**) from (+)-2′-demethoxygriseofulvin (**7**)[2] was planned. In this study, **6** and (−)-2′-demethyoxy-2′α-methyl-2′,3′-dihydrogriseofulvin (**8**) were synthesized from **7** by the method of Ito *et al.*[3] with some modifications (Chart 2). Since the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **8** revealed two kinds of methyl signals (0.94 and 1.06 ppm) due to the 2′- and 6′-positions, and the circular dichroism (CD) spectrum was as shown in Fig. 1a, **8** was suggested to be (−)-2′-demethoxy-2′α-methyl-2′,3′-dihydrogriseofulvin. On the other hand, 2′-demethoxy-2′β-methyl-2′,3′-dihydrogriseofulvin (**9**), as the standard sample, was synthesized from **6** by catalytic hydrogenation. The ^{1}H-NMR spectrum of **9** revealed one kind of methyl signal at 0.87 ppm which was assigned to the 2′- and 6′-methyl groups. Moreover, the coupling patterns of the 2′α,6′α-, 3′α,5′α- and 3′β,5′β-protons of **9** were broadly the same as those of (+)-griseofulvin (**3**) and (+)-2′-demethoxygriseofulvin (**7**), and their signals were detected at 2.33 ppm (doublet of doublets), 2.53 (multiplet), and 3.10



7 8 6 9

1) $(Me_3)_2CuLi$
2) Me_3SiCl
3) $Pd(OAc)_2$, *p*-BQ

Chart 2

(doublet of doublets), respectively. In addition, no CD spectrum was observed for **9**. The above results indicate that **9** has a conformation of the griseofulvin type[4] and both methyl groups have an equatorial configuration. On the other hand, the coupling patterns of the 2′α,6′α-, 3′α,5′α- and 3′β,5′β-protons of **8** were observed as widely multiple signals (2.42—2.86 ppm) and were clearly different from those of **9**. These findings led to the conclusion that the conformation of **8** is of the epigriseofulvin type.[4] Moreover, 500 MHz ^{1}H-NMR spectroscopy with selective proton decoupling established the assignments of all of the proton signals of **8**.

On the other hand, the ^{1}H-NMR spectrum of **6** showed two different signals due to the 2′- and 6′-methyl protons, and the CD spectrum (Fig. 1b) was observed to exhibit almost the same pattern as that of (+)-griseofulvin (**3**).[1b] The above results strongly supported the structure of **6**, which represents an important standard sample for the structural determination of the microbial transformation product.

Synthesis of 2′-Demethoxy-2′-methyldehydrogriseofulvin as the Substrate (Chart 3) Treatment of **6** with selenium oxide (SeO_2) or dichlorodicyanobenzoquinone (DDQ) did not afford the corresponding dehydrogenated product. We therefore planned a procedure for the dehydrobromination of the 5′-brominated compound of **6**. Treatment of **6** with pyridinium bromide perbromide ($PyHBr_3$) in chloroform afforded a mixture of 2′-bromomethyl-2′-demethoxygriseofulvin (**10**) and 5′α-bromo-2′-bromomethyl-2′-demethoxygriseofulvin (**11**), whose structures were determined by ^{1}H-NMR analysis, indicating that the desired 5′α-bromo derivative of **6** was not obtained.

Consequently, we attempted dehydrogenation of **8** with DDQ in dioxane, but no product was obtained. However, treatment of **8** with SeO_2 in *tert*-BuOH yielded a new product (**12**) whose structure was determined to be 2′-demethoxy-2′α-methyl-5′-hydroxy-2′,3′-dihydrodehydrogriseofulvin by high-resolution mass (high MS) analysis, elemental analysis, ^{1}H-, ^{13}C-, and two dimensional NMR experiments.

In order to synthesize **5** as the substrate, we therefore finally attempted the bromination of **8** with $PyHBr_3$ followed by dehydrobromination of the bromo derivative. The product was determined to be 2′-demethoxy-2′-methyldehydrogriseofulvin (**5**) by MS (molecular weight; *m/z* 334) and from the ^{1}H-NMR spectrum, in which a signal (6.23) assignable to the olefinic 3′- and 5′-H and a singlet signal at 1.80 ppm due to the 2′- and 6′-methyls on the olefinic carbon were observed.

Enzymatic Transformation of 2′-Demethoxy-2′-methyldehydrogriseofulvin to (+)-2′-Demethoxy-2′-methylgriseofulvin by a Cell-Free System of *Streptomyces cinereocrocatus* Incubation of (+)-2′-demethoxy-2′-methyldehydrogriseofulvin (**5**) was performed under the same conditions as described previously[5] for the conversion of (−)-dehydrogriseofulvin (**2**), and the transformation product was separated by column chromatography on silica gel and purified by recrystallization from MeOH. Analysis by MS (molecular weight; *m/z* 336) of the product indicated that hydrogenation of the substrate had occurred. The enzymatic transformation product was identified as (+)-2′-

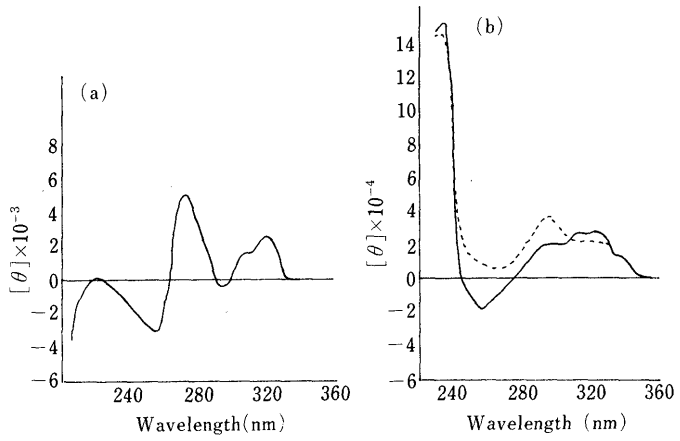


Fig. 1a. CD Spectrum of Compound **8**

Fig. 1b. CD Spectra of Chemically Synthesized Compound **6** (——) and the Enzymatic Transformation Product (**6**) (——), and (+)-Griseofulvin (**3**) (------)

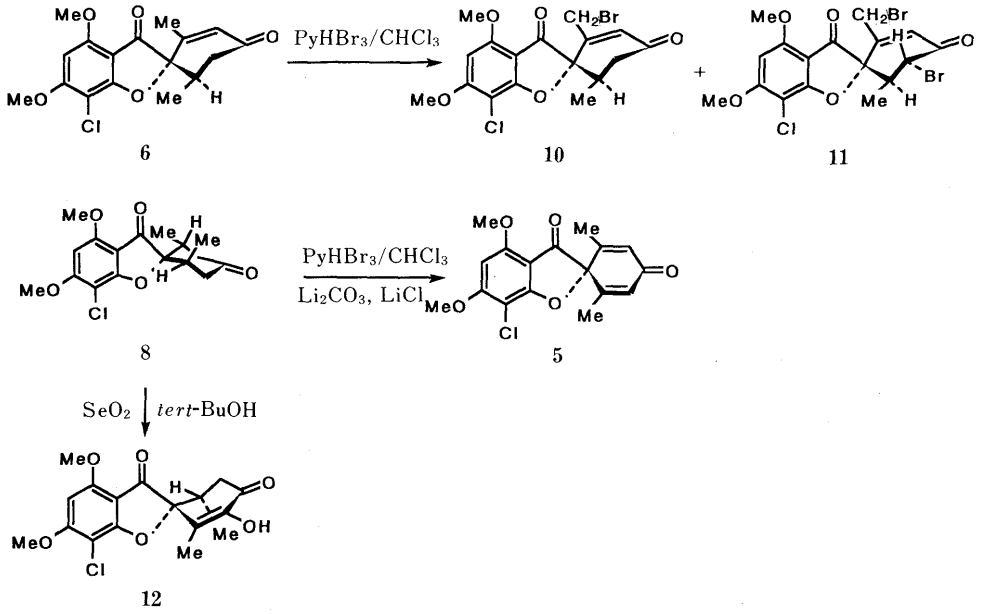


Chart 3

demethoxy-2′-methylgriseofulvin based on a comparison of the MS, ^{1}H-NMR, melting point and CD data with those of an authentic sample (**6**) synthesized chemically. Interestingly, the formation of **6** demonstrates that the cell-free system of *S. cinereocrocatus* has the capacity to reduce the 2′-methyl analog (**5**) of **1** as well as (−)-dehydrogriseofulvin (**1**), and that the 2′,6′-dimethyl compound (**5**) was transformed regio- and stereoselectively to the 2′-methyl analog of (+)-griseofulvin (**3**), which is one of the four possible stereoisomers.

In previous experiments, (−)- and (+)-dehydrogriseofulvin (**1** and **2**), 2′-propoxy analogs (**13** and **14**), and (−)-2′-demethoxydehydrogriseofulvin (**15**) have been regio- and enantiospecifically transformed to (+)-griseofulvin (**3**), the 2′-propoxy analog (**16**)[6] of (+)-griseofulvin, and (+)-2′-demethoxy-2′-methyl-6′β-demethylgriseofulvin (**17**), respectively, but (+)-2′-demethoxydehydrogriseofulvin (**4**) was transformed non-regiospecifically to afford two products (reduced products at the 2′,3′- or 5′,6′-positions).[2] This investigation demonstrates that the structurally symmetrical substrate in which both the 2′- and 6′-positions were substituted with a methyl group was regio- and stereoselectively hydrogenated by the cell-free system of *S. cinereocrocatus*.

Experimental

Apparatus All melting points were obtained on a Shimadzu MM2 micro-melting point apparatus, and are uncorrected. The ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured at 270 MHz and 67.8 MHz on a JEOL JNM-GX 270 FT NMR spectrometer, respectively. All ^{1}H- and ^{13}C-NMR data were recorded in deuteriochloroform, and chemical shifts are reported as parts per million downfield from Me_4Si ($\delta=0$). The coupling information of ^{13}C-NMR was obtained by employing a gated decoupling facility which permitted retention of the nuclear Overhauser effect. The ^{13}C-NMR spectra obtained in deuteriochloroform were referenced to the solvent signal, the known separations from Me_4Si being used in order to present the chemical shift data in the conventional manner. The abbreviations employed are as follows: s = singlet, d = doublet, br = broad, m = multiplet, dd = doublet of doublets, q = quartet. MS and high-resolution MS were recorded on a JEOL JMS-DX303 mass spectrometer at an ionizing potential of 70 eV. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh). The thin-layer chromatograph (TLC) plates (precoated TLC plates, Silica gel 60F-254, Merck) were visualized under UV light and/or by spraying with concentrated H_2SO_4 and heating on an electric heater. pH values were recorded on a LAB-O-MATE (Beckman-Toshiba, Ltd.).

(+)-2′-Demethoxy-2′-methylgriseofulvin (6) and (−)-2′-Demethoxy-2′α-methyl-2′,3′-dihydrogriseofulvin (8)[3] To a cold (0 °C) slurry of 3.8 g (20 mmol) copper (I) iodide in 40 ml of anhydrous ether, 25 ml (28 mmol) of a 1.1 M methyllithium ether solution was added. After the addition had been completed, 1.0 g (3.1 mmol) of 2′-demethoxygriseofulvin (**7**)[2] in 30 ml of ether was added, the resultant mixture was stirred for 15 min at 0 °C, and 3 ml of trimethylchlorosilane, 3.8 ml of triethylamine, and 1.2 ml of hexamethylphosphoramide were added. The mixture was then stirred at room temperature for 1 h, and diluted with three volumes of ether. The mixture obtained was washed successively with two 20 ml portions each of 5% HCl and 5% $NaHCO_3$, dried (Na_2SO_4) and concentrated *in vacuo* to give a yellow oil. This oil was immediately added to a clear solution of 571 mg (2.5 mmol) of $Pd(OAc)_2$ and 275 mg (2.5 mmol) of *p*-benzoquinone in 20 ml of acetonitrile, with stirring under nitrogen at room temperature. The resultant mixture was then stirred for 3 h. The reaction mixture was extracted with chloroform, and the chloroform solution was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to give an oil (2.3 g). The oil was found to consist of a mixture of two products (58 : 42) by gas liquid chromatography (GLC). The mixture in benzene was chromatographed on silica gel (10 g). 1) Elution with benzene–methylene chloride (60 : 40) afforded **8** (470 mg) as an oil. $[\alpha]_D^{24}$ −5° ($c=0.29$, $CHCl_3$). MS *m/z*: 338 (M^+) (for the ^{35}Cl-compound), 296, 255 (base peak). ^{1}H-NMR δ (ppm): 0.94 (3H, d, $J=6.7$ Hz, 2′α-CH_3), 1.06 (3H, d, $J=7.0$ Hz, 6′β-CH_3), 2.40—2.46 (1H, m, 6′α-H), 2.46 (1H, $J=13.7$, 13.7 Hz, 3′α-H), 2.48 (1H, ddd, $J=$ 14.0, 4.3, 1.5 Hz, 5′α-H), 2.58 (1H, m, 2′β-H), 2.73 (1H, ddd, $J=14.3$, 4.9, 1.5 Hz, 3′β-H), 2.83 (1H, br dd, $J=14.0$, 4.3 Hz, 5′β-H). 3.99 (3H, s, 4-OCH_3), 4.03 (3H, s, 6-OCH_3), 6.12 (1H, s, 5-H). CD ($c=1.34$ mg/ml, $CHCl_3$) $[\theta]^{24}$ (nm); 0 (347), 5040 (291) (positive maximum), 0 (283), −3280 (278) (negative maximum), −250 (238), −5800 (228). 2) Elution (410 mg) with benzene–methylene chloride (50 : 50) and recrystallization of the product from MeOH gave **6** as colorless needles, mp 178.5—179 °C, $[\alpha]_D^{24}$ +356.8° ($c=0.05$ $CHCl_3$). *Anal.* Calcd for $C_{17}H_{17}ClO_5$: C, 60.63; H, 5.09. Found: C, 60.64; H, 5.12. CD ($c=0.56$ mg/ml, $CHCl_3$) $[\theta]^{24}$ (nm); +157400 (232), 0 (243), −20000 (264) (negative maximum), 0 (271), +27200 (312), +26000 (318), +27200 (322), 0 (350). MS *m/z*: 336 (M^+) (for the ^{35}Cl-compound) (base peak), 294, 122. ^{1}H-NMR δ (ppm): 0.92 (3H, d, $J=6.6$ Hz, 6′-CH_3), 1.79 (3H, d, $J=1.7$ Hz, 2′-CH_3), 2.41 (1H, dd, $J=16.5$ and 13.9 Hz, 5′α-H), 2.86 (1H, m, 6′α-H). 3.06 (1H, dd, $J=16.5$ and 13.9 Hz, 5′β-H), 3.99 (3H, s, 4-OCH_3), 4.06 (3H, s, 6-OCH_3), 6.05 (1H, s, 3′-H), 6.14 (1H, s, 5-H). ^{13}C-NMR δ (ppm): 14.7 (br q, 2′-CH_3), 17.8 (dq, 6′-CH_3), 37.9 (br d, C-6′), 40.0 (br t, C-5′), 56.4 (q, C-6), 57.0 (q, C-4), 89.7 (d, C-5), 93.9 (m, C-1′), 97.3 (d, C-7), 105.5 (d, C-3a), 120.8 (ddd, C-3′), 155.9 (br dd, C-2′), 157.7 (dd, C-4), 164.7 (dd, C-6), 169.1 (s, C-7a), 102.9 (br d, C-3), 197.4 (br dd, C-4′).

2′-Demethoxy-2′β-methyl-2′,3′-dihydrogriseofulvin (9) A suspension of 5% palladium–charcoal catalyst (10 mg) in an ethyl acetate solution (5 ml) of **6** (20 mg) was shaken under a stream of hydrogen at atmospheric pressure and room temperature. The hydrogenation was stopped after 1.5 h. The catalyst was removed by filtration and the filtate was concentrated *in vacuo*. The residue (18 mg) was subjected to column chromatography on silica gel (10 g). Elution with benzene–methylene chloride (70 : 30) and recrystallization of the product from MeOH gave **9** as colorless needles, mp 235—236 °C, $[\alpha]_D^{24}$ 0° ($c=0.11$, $CHCl_3$). CD ($c=$ 1.08 mg/ml, $CHCl_3$): no CD was exhibited in the range of 230—360 nm. *Anal.* Calcd for $C_{17}H_{19}ClO_5$: C, 60.27; H, 5.65. Found: C, 60.21; H, 5.70. MS *m/z*: 338 (M^+) (for the ^{35}Cl-compound), 255 (base peak). ^{1}H-NMR δ (ppm): 0.87 (6H, d, $J=6.6$ Hz, 2′- and 6′-CH_3), 2.33 (2H, dd, $J=14.4$, 5.6 Hz, 3′α- and 5′α-H), 2.53 (2H, m, 2′α- and 6′α-H), 3.10 (2H, dd, $J=$ 14.5, 14.5 Hz, 3′β- and 5′β-H), 3.99 (3H, s, 4-OCH_3), 4.02 (3H, s, 6-OCH_3), 6.11 (1H, s, 5-H).

Reaction of 6 with $PyHBr_3$ A mixture of **6** (214 mg, 1 mmol) and $PyHBr_3$ (256 mg, 1.2 mmol) in chloroform (40 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*. Two spots were detected by TLC analysis. The residue in benzene was chromatographed on silica gel (50 g). Elution with benzene–methylene chloride (60 : 40) gave 2′-bromomethyl-2′-demethoxygriseofulvin (**10**) and 5′α-bromo-2′-bromomethyl-2′-demethoxygriseofulvin (**11**) as oils. **10**: MS *m/z*: 416 (M^+) (for the ^{35}Cl-compound), 334 (base peak), 416. ^{1}H-NMR δ (ppm): 0.92 (3H, d, $J=6.6$ Hz, 6′-CH_3), 2.44 (1H, dd, $J=17.3$, 5.0 Hz, 5′α-H), 2.87 (1H, m, 6′α-H), 3.09 (1H, dd, $J=17.2$, 13.9 Hz, 5′β-H), 3.90 (1H, d, $J=11.6$ Hz, $-CH_2Br$), 3.99 (3H, s, 4-OCH_3), 4.04 (3H, s, 6-OCH_3), 4.13 (1H, d, $J=11.6$ Hz, $-CH_2Br$), 6.16 (1H, s, 5-H), 6.33 (1H, s, 3′-H). **11**: MS *m/z*: 493 (M^+) (for the ^{35}Cl-compound), 414, 373, 214 (base peak). ^{1}H-NMR δ (ppm): 1.14 (3H, d, $J=6.6$ Hz, 6′-CH_3), 3.02 (1H, m, 6′α-H), 3.93 (1H, d, $J=12.2$ Hz, $-CH_2Br$), 4.00 (3H, s, 4-OCH_3), 4.06 (3H, s, 6-OCH_3), 4.11 (1H, d, $J=11.6$ Hz, $-CH_2Br$), 5.19 (1H, d, $J=12.5$ Hz, 5′β-H), 6.18 (1H, s, 5-H), 6.46 (1H, s, 3′-H).

Reaction of 8 with SeO_2 A solution of **8** (830 mg) and SeO_2 (866 mg) in *tert*-butanol (30 ml) was refluxed for 24 h. The hot reaction mixture was filtered and Darco-G-60 (800 mg) was added to the filtrate. The mixture was then filtered again after 20 min. After removal of the solvent, the residue in benzene was subjected to column chromatography on silica gel (100 g). Elution with benzene–methylene chloride (50 : 50) and recrystallization of the product from MeOH afforded 2′-demethoxy-2′α-methyl-5′-hydroxy-2′,3′-dihydrodehydrogriseofulvin (**12**) as colorless needles, mp 204—205 °C. *Anal.* Calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 58.02; H, 4.84. MS *m/z*: 352 (M^+) (for the ^{35}Cl-compound) (base peak), 255, 215. High resolution MS: Calcd for $C_{17}H_{17}ClO_6$: 352.0714. Found: 352.0340. ^{1}H-NMR δ (ppm): 0.92 (3H, br s, 2′α-CH_3), 1.75 (3H, s, 6′-CH_3), 2.72 (3H, br d, $J=2.0$ Hz, 2′β-5′α- and 5′β-H), 4.00 (3H, s, 4-OCH_3), 4.03 (3H, s, 6-OCH_3), 6.13 (1H, s, 5-H), 6.42 (1H, s, 5′-OH). ^{13}C-NMR δ (ppm): 11.4 (q, 6′-CH_3), 14.8 (br q, 2′-CH_3), 35.2 (dq, C-2′), 39.1 (ddt, C-3′), 56.4 (q, C-6), 57.0 (q, C-4), 89.4 (d, C-5), 93.6 (m, C-1′), 97.5 (d, C-7), 106.0 (d, C-3a), 121.7 (dd, C-6′), 147.2 (br dd, C-5′), 147.6 (dd, C-4), 164.7 (dd, C-6), 169.1 (s, C-7a), 192.9 (br s, C-3), 195.9 (s, C-4′).

Compound 5 A mixture of **8** (680 mg, 2.0 mmol) and $PyHBr_3$ (1.2 g, 4.0 mmol) in chloroform (140 ml) was refluxed for 2 h. The reaction

mixture was poured into a large volume of ice and water, and extracted with chloroform (300 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. A solution of the above residue (1.0 g), LiCl (160 mg), Li_2CO_3 (280 mg) and pyridine (16 ml) in dimethylformamide (DMF) (75 ml) was heated at 90 °C for 2 h. The reaction mixture was then poured into ice and water, and extracted with chloroform (300 ml × 3). The chloroform extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* (yield, 810 mg). The residue was chromatographed on silica gel (100 g) and eluted with benzene–methylene chloride (50 : 50). Recrystallization of the eluate from MeOH gave colorless needles of **5**, mp 239—241 °C (dec.), $[\alpha]_D^{24}$ 0° ($c=0.11$, $CHCl_3$). CD ($c=0.53$ mg/ml, $CHCl_3$): no CD was exhibited in the range of 230—360 nm. *Anal.* Calcd for $C_{17}H_{15}ClO_5$; C, 60.99; H, 4.52. Found: C, 60.94; H, 4.51. MS *m/z*: 334 (M^+) (for the ^{35}Cl-compound) (base peak), 197. ^{1}H-NMR δ (ppm): 1.80 (6H, s, 2′- and 6′-CH_3), 3.99 (3H, s, 4-OCH_3), 4.06 (3H, s, 6-OCH_3), 6.17 (1H, s, 5-H), 6.23 (2H, br s, 3′- and 5′-H).

Transformation of 5 to 6 by the Cell-Free System of *Streptomyces cinereocrocatus* The incubation and separation were carried out essentially as described previously[5] except that 22 mg of **5** was used as the substrate in the cell-free system[5] (220 ml) of *S. cinereocrocatus* NRRL 3443. The residue (14 mg) from the incubation mixture consisted of **6** (82% yield, by ^{1}H-NMR analysis) and the starting material (**5**). Column chromatography of the residue on silica gel (30 g) and recrystallization of the product from MeOH afforded **6**, mp 178—178.5 °C. *Anal.* Calcd for $C_{17}H_{17}ClO_5$: C, 60.63; H, 5.09. Found: C, 60.61; H, 5.14. $[\alpha]_D^{24}$ +358.4° ($c=0.06$, $CHCl_3$). CD ($c=0.63$ mg/ml, $CHCl_3$) $[\theta]^{24}$ (nm); +157700 (232), 0 (243), −19800 (264) (negative maximum), 0 (271), +27400 (312), +26100 (318), +28000 (322), 0 (350). The MS and ^{1}H-NMR data were identical with those of the standard sample (**6**) synthesized chemically.

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References

1) *a*) Y. Sato, T. Oda, and H. Saitô, *J. Chem. Soc., Chem. Commun.*, **1977**, 415; *b*) *Idem*, *Chem. Pharm. Bull.*, **29**, 2313 (1981).
2) T. Oda and Y. Sato, *Chem. Pharm. Bull.*, **31**, 934 (1983); *idem*, *ibid.*, **31**, 3446 (1983).
3) Y. Ito, T. Hirao, and T. Saegusa, *J. Org. Chem.*, **43**, 1011 (1978).
4) A. Itai, Y. Iitaka, S. Nakamura, T. Oda, and Y. Sato, *Chem. Pharm. Bull.*, **33**, 158 (1985).
5) T. Oda and Y. Sato, *Chem. Pharm. Bull.*, **33**, 1077 (1985).
6) Y. Sato and T. Oda, *Heterocycles*, **17**, 171 (1982).