MICROBIAL TRANSFORMATION OF 2'-PROPOXY ANALOGS OF (-)- AND (+)-DEHYDROGRISEOFULVIN AND (+)-2'-DEMETHOXYDEHYDROGRISEOFULVIN BY STREPTOMYCES CINEREOCROCATUS

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Abstract —— By the fermentation of *Streptomyces cinereocrocatus*, 2'-propoxy analogs of (-)- and (+)-dehydrogriseofulvin were both converted into the corresponding analog of (+)-griseofulvin and the same treatment of (+)-2'-demethoxydehydrogriseofulvin afforded (+)-2'-demethoxygriseofulvin and (+)- and (-)-2'-demethoxy-2',3'-dihydrodehydrogriseofulvin, indicating that the (+)-substrates were isomerized into the corresponding (-)-enantiomers and subsequently transformed to the reduction products.

The microbial transformation of (-)-dehydrogriseofulvin to (+)-griseofulvin was initially investigated by Andres and his co-workers[1] using *Streptomyces cinereocrocatus* NRRL 3443. Since then, we have demonstrated that (-)- and (+)-dehydrogriseofulvin are both transformed mainly into (+)-griseofulvin by *Streptomyces* species including *Streptomyces cinereocrocatus* and the stereochemistry of the microbial reduction is successfully elucidated by ^{2}H NMR spectroscopy.[2,3]
We describe the following studies which clarify that the microbial transformations of 2'-propoxy analogs (**1** and **2**) of (-)- and (+)-dehydrogriseofulvin and (+)-2'-demethoxydehydrogriseofulvin (**3**) by *Streptomyces cinereocrocatus* take place directly or after isomerizations with hydrogenations depending on 2'-substituents of (-)- and (+)-dehydrogriseofulvin analogs. 2'-Propoxy analogs (**1** and **2**) were used as the substrates in place of 2'-ethoxy analogs, since in the microbial transformation the former will give more informations connected with the replacement of the 2'-methoxy group of (-)- and (+)-dehydrogriseofulvin than the latter.
Firstly, the substrates (**1** and **2**) were synthesized as follows. Reaction of 2'-propoxy analog (**4**)[4] of (+)-griseofulvin in the presence of selenium dioxide in

tert-butanol, followed by silica gel column chromatography afforded 2'-propoxy analog (**1**) of (-)-dehydrogriseofulvin [PMR δ ($CDCl_3$) 0.79 (3H, t, *J* = 7 Hz, 2'-$OCH_2CH_2\underline{CH_3}$), 1.60 (2H, sextet, *J* = 7 Hz, 2'-$OCH_2\underline{CH_2}CH_3$), 1.79 (3H, bs, 6'-$CH_3$), 3.79 (2H, t, *J* = 7 Hz, 2'-$O\underline{CH_2}CH_2CH_3$), 4.00 (3H, s, 4-$OCH_3$), 4.07 (3H, s, 6-$OCH_3$), 5.66 (1H, bs, 3'-H), 6.18 (1H, s, 5-H), 6.20 (1H, bs, 5'-H)].[5] The same dehydrogenation reaction of 2'-propoxy analog of (+)-epigriseofulvin which was obtained by alkylation of (+)-epigriseofulvic acid with diazopropane afforded 2'-propoxy analog (**2**) of (+)-dehydrogriseofulvin, which showed the same PMR spectrum but exhibited opposite optical properties[6] compared with those of **1**. Catalytic hydrogenation of **2** yielded 2'-propoxy analog (**5**)[7] of (-)-griseofulvin.

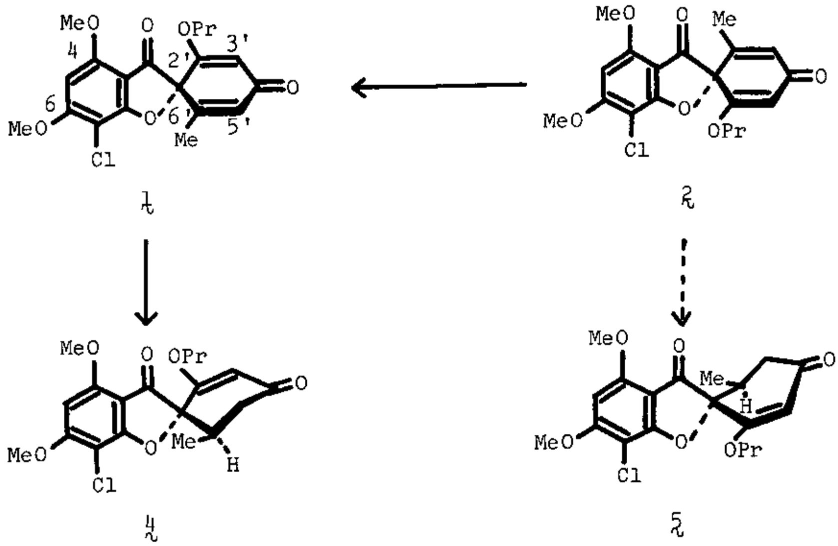
In connection with the previous studies[3], the microbial treatment of **1** by *S. cinereocrocatus* under previously described conditions gave **4** as the reduction product and the recovered material, which were separated by silica gel column chromatography. On the other hand, the same microbial treatment of **2** was performed and its results were compared with those of the enantiomer (**1**). The results indicate that reactions of **1** proceed more rapidly than those of **2** by comparisons of the yields of the reduction product and the recovered material(s). Moreover, the comparisons of susceptibilities to microbial transformations between (-)- and (+)-dehydrogriseofulvin and their 2'-propoxy analogs indicate that the propoxy analogs are less

Table I. Yields of Reduction Products and Relative Ratios of (+)- and (-)-Enantiomers of Recovered Substrates

Substrates	Reduction products: Yields (%)	Recovered substrates: Yields (%)	Relative ratios (%) of (+)-	Relative ratios (%) of (-)-
(-)-Dehydrogriseofulvin*	88	0	——	——
2'-Propoxy analog of (-)-dehydrogriseofulvin	17	24	0	100
(+)-Dehydrogriseofulvin*	31	15	100	0
2'-Propoxy analog of (+)-dehydrogriseofulvin	3	57	96	4
2'-Propoxy analog of (±)-dehydrogriseofulvin	20	25	72	28

* The experiments using these substrates were performed as controls for the corresponding (-)- and (+)-2'-propoxy analogs. And their reduction products were the same optically pure (+)-griseofulvin.[3]

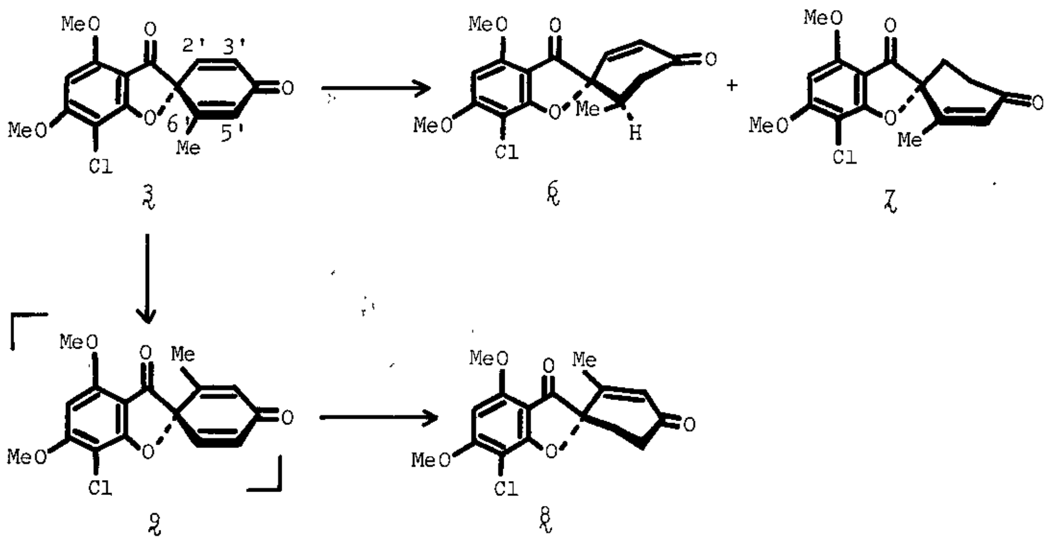
transformed by S. cinereocrocatus (see Table I).[8] Furthermore, the relative ratios of these compounds clearly demonstrate that both substrates were transformed into the optically pure 2'-propoxy analog of (+)-griseofulvin in spite of a fact that recovered dehydrogriseofulvin analogs were a mixture of (+)- and (−)-enantiomers in the microbial treatment of 2'-propoxy analog (2) of (+)-dehydrogriseofulvin. Further, it is of importance to notice that in the microbial treatment by S. cinereocrocatus 2'-propoxy analog (2) of (+)-dehydrogriseofulvin was not transformed into the corresponding hydrogenated product (5). These results are summarized in Scheme 1.



Scheme 1

In order to extensively elucidate the microbial transformation, (+)-2'-demethoxydehydrogriseofulvin was synthesized as follows. A solution of (+)-2'-demethoxygriseofulvin (6)[9] and pyridinium hydrobromide perbromide[10] in chloroform was reacted under reflux for 2 hr to give a 40:60 mixture (g.l.c.) of 3'-bromo-2'-demethoxygriseofulvin[11] and 5'α-bromo-2'-demethoxygriseofulvin[12], which was separated by repeated recrystallization from methanol and silica gel column chromatography. Subsequent dehydrobromination[13] of the 5'α-bromo derivative with LiCl and Li_2CO_3 in DMF containing pyridine at 100°C for 24 hr yielded (+)-2'-demethoxydehydrogriseofulvin (3)[14] [PMR δ ($CDCl_3$) 1.82 (3H, bs, 6'-CH_3), 3.98 (3H, s, 4-OCH_3), 4.08 (3H, s, 6-OCH_3), 6.18 (1H, s, 5-H), 6.29 (1H, m, 3'-H), 6.42 (1H, d, J = 2 Hz, 5'-H), 6.53 (1H, d, J = 10 Hz, 2'-H)].

The microbial treatment of (+)-2'-demethoxydehydrogriseofulvin (3) by S. cinereocrocatus for 12 hr under the same conditions described above afforded (+)-2'-demethoxygriseofulvin (6) (12%) and a mixture of (-)- and (+)-2'-demethoxy-2',3'-dihydrodehydrogriseofulvin (7 and 8)[15] (8%), whose relative ratio was calculated as 19:81 from the value[16] of its circular dichroism. When the incubation period was shortened by 3 hr, 6 and a mixture[17] of 7 and 8 were obtained in 3 and 8% yields, respectively, with 52% yield of the recovered 3. These results are summarized in Scheme 2, which indicates that the microbial reductions of 3 proceed more preferencially in 5',6'- than 2',3'-double bond. Furthermore, the formation of (+)-2'-demethoxy-2',3'-dihydrogriseofulvin (8) suggests that the microorganism has the abilities of the isomerization of the substrate (3) into the enantiomer (9) and of the subsequent reduction of the latter.



Scheme 2

Hence, we conclude that in the treatment with S. cinereocrocatus, the analogs of (-)- and (+)-dehydrogriseofulvin which have or have not substituents at 2'-position are reduced directly or after isomerization into the corresponding enantiomers, yielding (+)- and/or (-)-dihydro compound(s) as the transformation products.

ACKNOWLEDGEMENT

Thanks are due to Dr. T. G. Pridham of ARS Culture Collection Research for providing the strain of Streptomyces cinereocrocatus and to Professor S. Okuda of the Institute of Applied Microbiology, University of Tokyo, for elemental analyses.

REFERENCES AND NOTES

1. W. W. Andres, W. J. McGahren, and M. P. Kunstmann, Tetrahedron Letters, 1969, 3777.

2. Y. Sato, T. Oda, and H. Saitô, J.C.S.,Chem. Comm., 1977, 415.

3. Y. Sato, T. Oda, and H. Saitô, Chem. Pharm. Bull., 1981, **29**, 2313.

4. L. A. Duncanson, J. F. Grove, and P. W. Jeffe, J. Chem. Soc., 1958, 2929.

5. The molecular ellipticity [θ] (c 1.0 mg/ml, $CHCl_3$): $[\theta]_{365}$ -560, $[\theta]_{335}$ -7000, $[\theta]_{328}$ -5600, $[\theta]_{298}$ -28350, $[\theta]_{290}$ 0, $[\theta]_{280}$ +19600, $[\theta]_{260}$ 0, $[\theta]_{257}$ -2100, $[\theta]_{255}$ 0, $[\theta]_{245}$ +18200, $[\theta]_{241}$ 0, $[\theta]_{235}$ -40600; $[\alpha]_D^{21}$ -57.5° (c 0.56, acetone).

6. The molecular ellipticity [θ] (c 1.0 mg/ml, $CHCl_3$): $[\theta]_{365}$ +490, $[\theta]_{335}$ +7000, $[\theta]_{328}$ +5600, $[\theta]_{298}$ +26600, $[\theta]_{290}$ 0, $[\theta]_{280}$ -19600, $[\theta]_{260}$ 0, $[\theta]_{257}$ +1750, $[\theta]_{255}$ 0, $[\theta]_{245}$ -17500, $[\theta]_{241}$ 0, $[\theta]_{235}$ +39900; $[\alpha]_D^{21}$ +56.0° (c 0.51, acetone).

7. PMR and mass spectra were identical with those of **4**. However, the CD and optical rotation showed the opposite values.

8. The microbial transformations of (-)- and (+)-dehydrogriseofulvin and its 2'-propoxy analogs were performed in the incubation periods of 5 hr, one day, 3 days, and 5 days. Table I shows, however, the results of 3-day's incubations.

9. T. P. C. Mulholland, J. Chem. Soc., 1952, 3994.

10. C. Djerassi and C. R. Scholz, J. Am. Chem. Soc., 1948, **70**, 417.

11. PMR ($CDCl_3$) δ 0.92 (3H, d, *J* = 6 Hz, 6'-CH_3), 2.5-3.3 (3H, m, 5'α, 5'β and 6'α-H), 3.97 (3H, s, 4-OCH_3), 4.02 (3H, s, 6-OCH_3), 6.13 (1H, s, 5-H), 7.01 (1H, s, 2'-H).

12. PMR ($CDCl_3$) δ 1.13 (3H, d, *J* = 6 Hz, 6'-CH_3), 3.03 (1H, d.d, *J* = 6 and 13 Hz, 6'α-H), 3.97 (3H, s, 4-OCH_3), 4.03 (3H, s, 6-OCH_3), 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).

13. R. P. Holysz, J. Am. Chem. Soc., 1953, **75**, 4432.

14. The molecular ellipticity [θ] (c 1.0 mg/ml, $CHCl_3$): $[\theta]_{370}$ -480, $[\theta]_{343}$ -3360, $[\theta]_{333}$ -800, $[\theta]_{330}$ -900, $[\theta]_{327}$ 0, $[\theta]_{300}$ +9600, $[\theta]_{284}$ 0, $[\theta]_{270}$ -5440, $[\theta]_{263}$ 0, $[\theta]_{255}$ +6720, $[\theta]_{248}$ +4800, $[\theta]_{238}$ +43520.

15. Authentic sample of **7** was synthesized by dehydrogenation of (-)-2'-demethoxy-dihydrogriseofulvin(cf. A. W. Dawkins and T. P. C. Mulholland, J. Chem. Soc., 1959, 1826) with selenium dioxide in *tert*-butanol. Compound **7**: PMR ($CDCl_3$) δ 1.80 (3H, bs, 6'-CH_3), 2.3-2.8 (4H, m, 2'- and 3'-H), 4.03 (3H, s, 4-OCH_3),

4.07 (3H, s, 6-OCH_3), 6.14 (1H, bs, 5'-H), 6.20 (1H, s, 5-H); The molecular ellipticity [θ] (c 1.0 mg/ml, $CHCl_3$): $[\theta]_{370}$ -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. The comparison of CD data of related compounds suggests that the conformation of **7** is as shown in Scheme 2.

16. The molecular ellipticity [θ] (c 1.0 mg/ml, $CHCl_3$): $[\theta]_{370}$ 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.
17. The relative ratio of **7** and **8** was 28:72 and the recovered **3** was optically pure on the basis of their CD data.

Received, 21th August, 1981