Organic Process Research & Development **2002**, *6*, 482−487

Hydroxylation of Mutilin by *Streptomyces griseus* and *Cunninghamella echinulata*

Ronald L. Hanson,*,† James A. Matson,‡ David B. Brzozowski,† Thomas L. LaPorte,† Dane M. Springer,§ and Ramesh N. Patel†

Process Research and Development, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, One Squibb Drive, New Brunswick, New Jersey 08903, U.S.A., Department of Natural Products Chemistry, and Department of Anti-infective Chemistry, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492, U.S.A.

Abstract:

Biotransformation of mutilin and pleuromutilin by microbial cultures was investigated to provide a source of 8-hydroxymutilin or 8-hydroxypleuromutilin. LC/MS analysis of culture broths showed that several strains gave M+16 products from mutilin and one culture gave an M+16 product from pleuromutilin, suggesting addition of oxygen. Biotransformation products were extracted from culture broths with ethyl acetate, dried, and purified by chromatography on silica gel. *Streptomyces griseus* strains SC 1754 and SC 13971 (ATCC 13273) converted mutilin to (8*S*)-, (7*S*)-, and (2*S*)-hydroxymutilin. *Cunninghamella echinulata* SC 16162 (NRRL 3655) gave (2*S*)-hydroxymutilin or (2*R*)-hydroxypleuromutilin from biotransformation of mutilin or pleuromutilin, respectively. The biotransformation of mutilin by *S. griseus* strain SC 1754 was scaled up in 15-, 60-, and 100-L fermentations to produce a total of 49 g of (8*S*)-hydroxymutilin (BMS-303786), 17 g of (7*S*)-hydroxymutilin (BMS-303789) and 13 g of (2*S*)-hydroxymutilin (BMS-303782) from 162 g of mutilin.

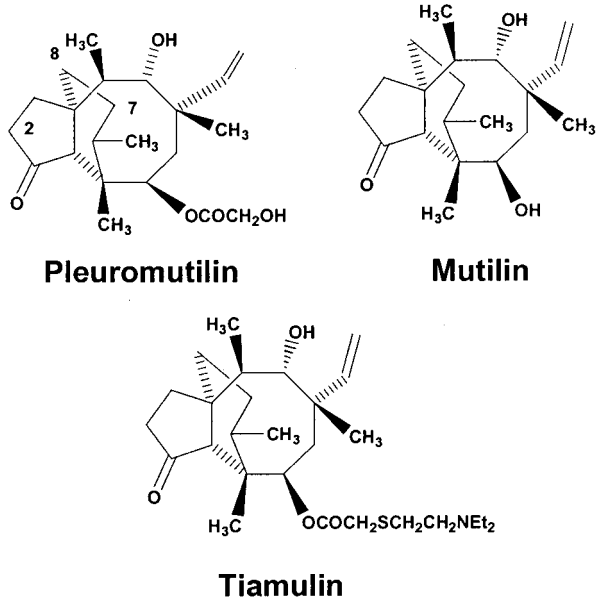

Figure 1. **Structures of pleuromutilin, mutilin, and tiamulin.**

Introduction

Pleuromutilin is an antibiotic from *Pleurotus* or *Clitopilus* basidiomycete strains which kills mainly Gram-positive bacteria and mycoplasms (Figure 1). A more active semisynthetic analogue, tiamulin, has been developed for the treatment of animals and poultry and has been shown to bind to prokaryotic ribosomes and inhibit protein synthesis.[1,2] Metabolism of pleuromutilin derivatives results in hydroxylation by microsomal cytochrome P-450 at the 2- or 8-position and inactivates the antibiotics.[3−5] 8-Hydroxymutilin has been shown to be a metabolite produced from tiamulin by swine.[6] Modification of the 8-position of pleuromutilin and analogues is of interest as a means of preventing the metabolic hydroxylation. Microbial hydroxylation at the 8-position of pleuromutilin or mutilin would provide a functional group at this position to allow further chemical modification at this site to avoid metabolic hydroxylation when the compounds are administered to animals. The target analogues would maintain the biological activity of the parent compounds but not be susceptible to metabolic inactivation. This report describes screening experiments to identify organisms able to carry out the hydroxylation, growth of the cultures and hydroxylation of mutilin in pilot-plant fermentors, and isolation and characterization of the hydroxylation products.

Results

Screening. Cultures were screened for 8-hydroxylation or other biotransformation of mutilin and pleuromutilin on a nutrisoy/glucose/yeast extract medium known to be favorable for hydroxylation.[7] From about 100 cultures, three were found which metabolized all the mutilin and gave M+16 peaks by LC/MS analysis. One of these cultures also

* Corresponding author. Telephone and fax: 1-732-519-2797. E-mail: ronald.hanson@bms.com.
† Process Research and Development.
‡ Department of Natural Products Chemistry.
§ Department of Anti-infective Chemistry.

(1) Hogenauer, G. In *Antibiotics*; Hahn, F. E., Ed.; Springer-Verlag: New York, 1979; Vol. V/Part 1, pp 344−360.
(2) Erkel, G. In *Fungal Biotechnology*; Anke, T., Ed.; Chapman and Hall: Weinheim, 1997; pp 128−135.
(3) Berner, H.; Schulz, G.; Fischer, G. *Monatsh. Chem.* **1981**, *112*, 1441.
(4) Berner, H.; Vyplel, H.; Schulz, G.; Stuchlik, P. *Tetrahedron* **1983**, *39*, 1317.
(5) Berner, H.; Vyplel, H.; Schulz, G.; Stuchlik, P. *Monatsh. Chem.* **1983**, *114*, 1125.
(6) Markus, J. R.; Sherma, J. *J. AOAC Int.* **1993**, *76*, 459.
(7) Smith, R. V.; Rosazza, J. P. *Arch. Biochem. Biophys.* **1974**, *161*, 551.

Published on Web 05/14/2002
10.1021/op020028q CCC: $22.00

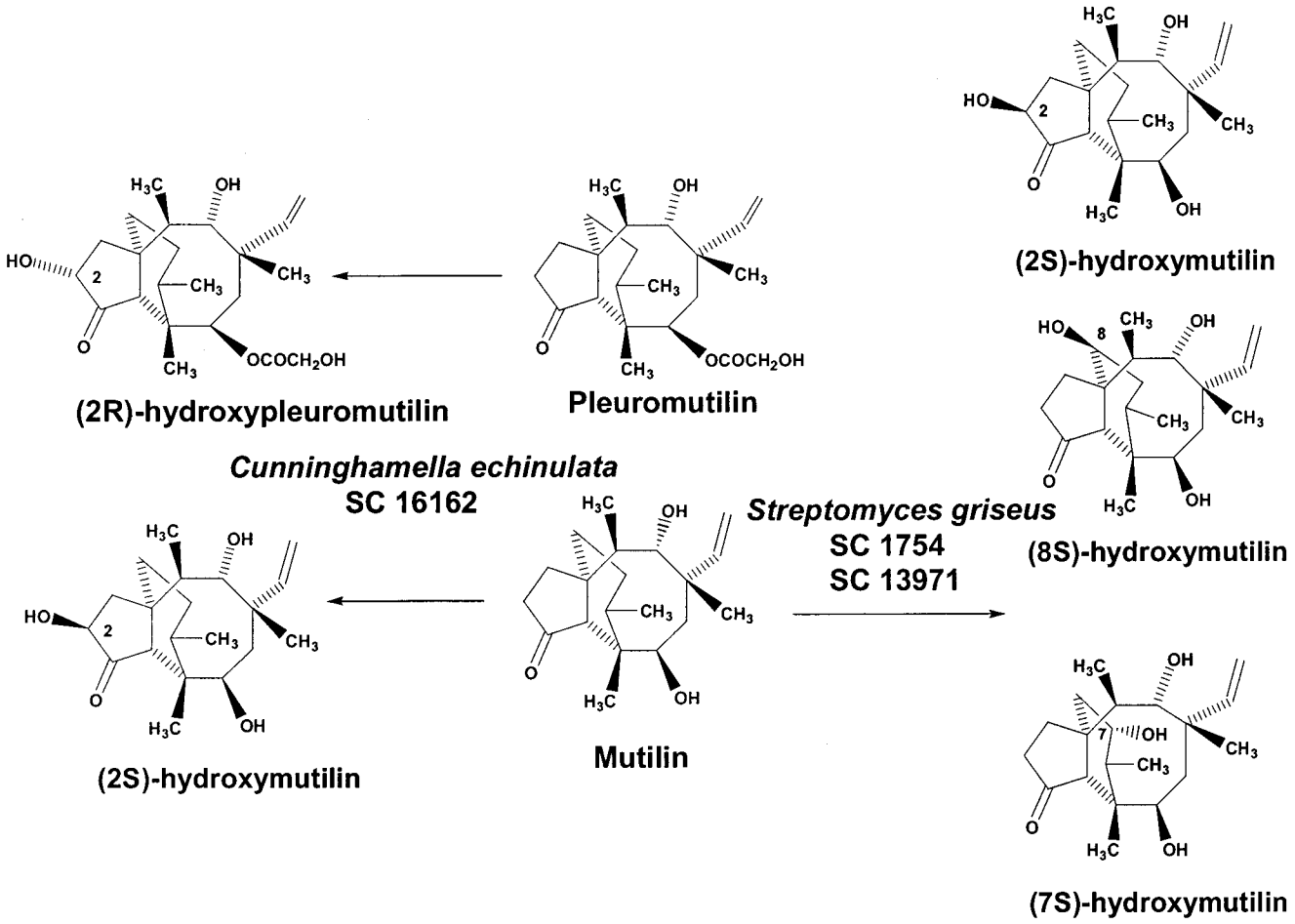

Figure 2. **Structures of biotransformation products.**

metabolized all the pleuromutilin and gave an M+16 molecular weight peak on LC/MS. The molecular weight increase could indicate hydroxylation, epoxidation, or a Baeyer−Villiger oxidation. These three cultures were used for preparative batches. Four additional cultures (known to hydroxylate compactin) decreased the mutilin concentration by about half and gave M+16 peaks by LC/MS analysis. These products were not characterized further.

Biotransformation in Shake Flasks. Biotransformation batches (250 mg of mutilin) were carried out using 500 mL of each culture. The broths were extracted with ethyl acetate, the solvent was removed, and the products were isolated from the residue by silica gel chromatography. Structures were determined by NMR and X-ray analysis. The filamentous fungus *Cunninghamella echinulata* SC16162 gave (2*S*)-hydroxymutilin from mutilin and (2*R*)-hydroxypleuromutilin from biotransformation of pleuromutilin. *Streptomyces griseus* strains SC 1754 and SC 13971 produced the desired product (8*S*)-hydroxymutilin as well as (7*S*)-hydroxymutilin (also of interest) and (2*S*)-hydroxymutilin. Structures of the biotransformation products are shown in Figure 2.

In a flask study, 0.5% glucose was compared with 2% glycerol as a carbon source for hydroxylation of 0.5, 1.0, and 2.0 mg/mL mutilin. Higher yields were produced with 0.5% glucose than with 2% glycerol. A further experiment was done in shake flasks containing medium B with various glucoseconcentrations. The highest yields of (7*S*)- and (8*S*)-hydroxymutilin were obtained with 1−1.5% glucose.

Biotransformation in Fermentors. Initial scale-up to 7.5 g of mutilin in 15 L of medium A with the two *S. griseus* strains gave incomplete conversion (Table 1, batches 1 and 2). A modified medium with 2% nutrisoy and 0.5% glucose (medium B) was then found to give faster hydroxylation in flasks for strain SC 1754, and this strain and medium were used for further batches.

A 15-L batch (3) and a 60-L batch (4) of *Streptomyces* sp. SC 1754 were used to hydroxylate 7.5 and 30 g of mutilin, respectively, and the tank results were similar to the previous flask results. A 100-L batch (5) of *Streptomyces* sp. SC 1754 was used to hydroxylate 50 g of mutilin. Analytical results for this batch before isolation of the products indicated 10.4 g of (8*S*)-hydroxymutilin and 2.6 g of (7*S*)-hydroxymutilin. The conversion was slow and incomplete in this batch, although the fermentation parameters measured appeared similar to those of the previous two batches. Whenever a tank performed poorly, a 50-mL sample of it incubated in a shake flask gave a similar conversion, suggesting that variation in the medium such as a difference in the amount of inducers in the toasted nutrisoy could be responsible. In subsequent batches, a sample of the toasted nutrisoy was tested in flasks before use in fermentors.

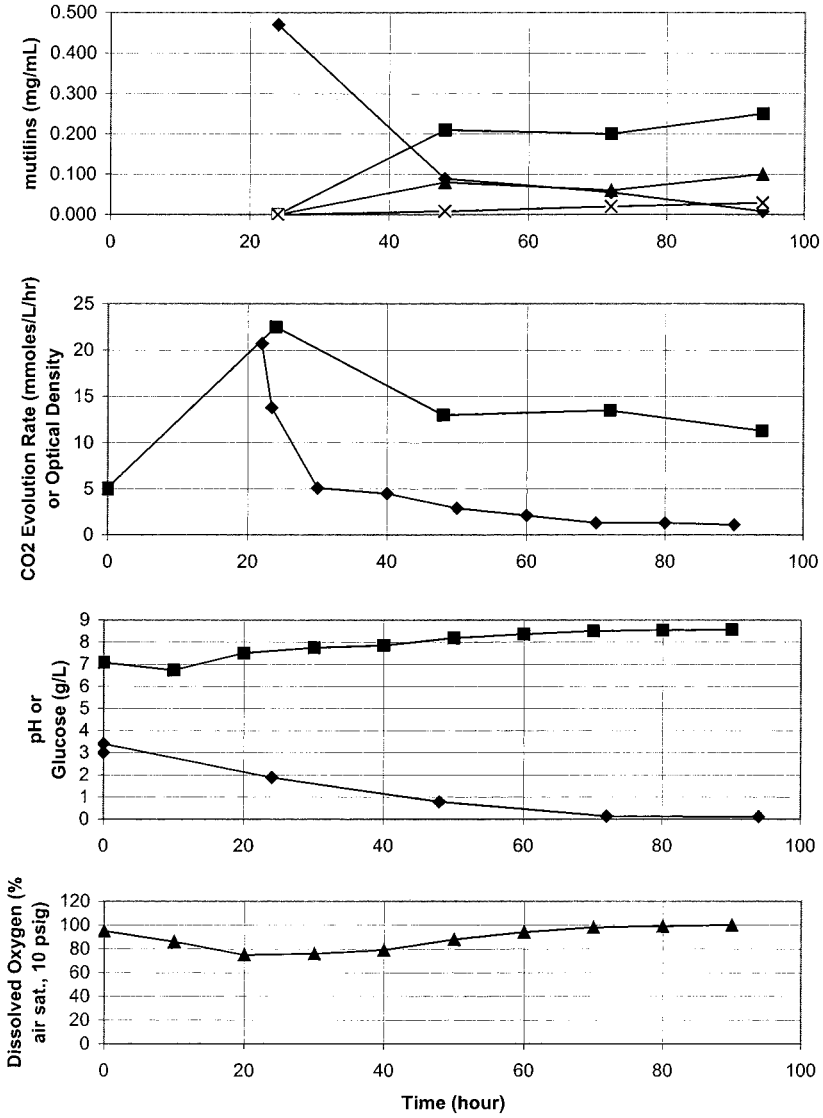

Figure 3. **Production of hydroxymutilins in a 60-L batch fermentor. The fermentation/biotransformation was conducted as described in the Experimental Section. (a) Mutilin hydroxylation in batch 6: (◆) mutilin, (■) 8-hydroxymutilin, (▲) 7-hydroxymutilin, (×) 2-hydroxymutilin. (b) (◆) carbon dioxide evolution rate, (■) optical density. (c) (■) pH, (◆) glucose, (d) (▲) dissolved oxygen.**

Table 1. **Summary of Pilot Plant Batches**

batch	strain SC no.	medium	input mutilin (g)	final mutilin (g)	(8*S*)-hydroxymutilin (g)	(7*S*)-hydroxymutilin (g)	(2*S*)-hydroxymutilin (g)
1	1754	A	7.5	2.550	0.735	1.650	0.075
2	13971	A	7.5	1.560	1.515	1.200	0.885
3	1754	B	7.5	0.616	2.800	0.742	0.588
4	1754	B	30.0	3.180	10.200	3.000	0.840
5	1754	B	50.0	19.250	10.430	2.555	2.730
6	1754	B	30.0	0.440	13.750	5.500	1.595
7	1754	B	30.0	8.250	9.900	2.750	6.050
	totals:		162.5	35.846	49.330	17.397	12.763

A 60-L batch (6) of *Streptomyces* sp. SC 1754 was used to hydroxylate 30 g of mutilin. Analytical results for the batch before isolation of the products indicated yields of 13.75 g of (8*S*)-hydroxymutilin and 5.50 g of (7*S*)-hydroxymutilin. The time course for biotransformation of mutilin in batch 6 is shown in Figure 3a, CO_2 and optical density are shown in 3b, pH and glucose in 3c, and dissolved oxygen in 3d.

Another 60-L batch (7) of *Streptomyces* sp. SC 1754 was used to hydroxylate 30 g of mutilin. Analytical results for the batch before isolation of the products indicated yields of 9.90 g of (8*S*)-hydroxymutilin and 2.75 g of (7*S*)-hydroxymutilin. There were no obvious differences between the last two batches that correlate with the lower yield from batch 7. A summary of analytical results for the batches before isolation of the products is shown in Table 1. A total of 49 g of (8*S*)-hydroxymutilin, 17 g of (7*S*)-hydroxymutilin, and 12.8 g of (2*S*)-hydroxymutilin (HPLC yields of crude products) were produced in pilot-plant fermentors.

Discussion

Microbial hydroxylation of mutilin has not been described previously, although the production of 8-hydroxymutilin by *Clitopilus* species has been reported.[8] Screening experiments indicated that two strains of *S. griseus* converted mutilin to 8-hydroxy- as well as 7-hydroxy- and 2-hydroxymutilin. *S. griseus* strains grown on media containing soy flour are known to carry out hydroxylation of various compounds as well as other oxidative biotransformations.[9] The isoflavonoid genestein has been identified as a component of soy flour acting as an inducer of a cytochrome P-450 monooxygenase.[10] Sariaslani and co-workers have purified and characterized the three proteins of the *S. griseus* ATCC 13273 soy flour inducible system, ferredoxin reductase,[11] ferredoxin$_{soy}$,[12] and P-450$_{soy}$.[9] The genes for ferredoxin$_{soy}$ and P-450$_{soy}$ have been cloned and P-450$_{soy}$ was expressed in *S. lividans* by this group.[13] It is likely that the same monooxygenase enzyme system characterized by these workers is responsible for mutilin hydroxylation by *S. griseus* strains SC 1754 and SC 13971. The hydroxylations of mutilin at the 7- and 8-positions introduce a functional group at these sites which allows further modification of the molecule. Of particular interest will be further modification of the 8-position (such as substitution of 8-fluoro- for the 8-hydroxyl substituent) to prevent 8-hydroxylation by the liver, leading to inactivation of the antibiotic.

Pleuromutilin was converted slightly to M+16 products by the *S. griseus* strains, but the products were not characterized. The killing of the bacteria by the antibiotic appeared to limit the conversion. *C. echinulata* SC 16162, a filamentous fungus not susceptible to the antibiotic, gave (2*S*)-hydroxymutilin or (2*R*)-hydroxypleuromutilin from biotransformation of mutilin or pleuromutilin, respectively. Thus, the presence or absence of the glycolate group changed the stereochemistry of the hydroxylation at the 2-position. Hydroxylation of various compounds by cytochrome P-450 monooxygenase from *C. echinulata* and other *Cunninghamella* species has been described,[14−19] although the enzymes involved have not been isolated and characterized.

(8) Palma, N.; Knauseder, F. In *Third European Congress of Biotechnology*; Verlag Chemie: Weinheim, 1984; Vol. 1, pp 533−542.
(9) Trower, M. K.; Sariaslani, F. S.; O'Keefe, D. P. *J. Bacteriol*. **1989**, *171*, 1781.
(10) Sariaslani, F. S.; Kunz, D. A. *Biochem. Biophys. Res. Commun*. **1986**, *141*, 405.
(11) Ramachandra, M.; Ramnath, S.; Emptage; M. H.; Sariaslani, F. S. *J. Bacteriol*. **1991**, *73*, 7106.
(12) Trower, M. K.; Emptage, M. H.; Sariaslani, F. S. *Biochim. Biophys Acta* **1990**, *1037*, 281.
(13) Trower, M. K.; Lenstra, R.; Omer, C.; Bucholz, S. E.; Sariaslani, F. S. *Mol. Microbiol.* **1992**, *6*, 2125.
(14) Schocken, M. J.; Mao, J.; Schabacker, D. J. *J. Agric. Food Chem*. **1997**, *45*, 3647.
(15) Zhang, D.; Hansen, E. B., Jr.; Deck, J.; Heinze, T. M.; Henderson, A.; Korfmacher, W. A.; Cerniglia, C. E. *Xenobiotica* **1997**, *27*, 301.
(16) Ferris, J. P.; Fasco, M. J.; Stylianopoulou, F. L. *Arch. Biochem. Biophys.* **1973**, *156*, 97.
(17) Cerniglia, C. E.; Fu, P. P.; Yang, S. K. *Biochem. J.* **1983**, *216*, 377.
(18) Cerniglia, C. E.; Lambert, K. J.; Miller, D. W.; Freeman, J. P. *Appl. Environ. Microbiol.* **1984**, *47*, 111.
(19) Zhang, D.; Evans, F. E.; Freeman, J. P.; Duhart, B., Jr.; Cerniglia, C. E. *Drug Metab. Dispos.* **1995**, *23*, 1417.

Experimental Section

Screen for Biotransformation. Growth medium A contained 0.5% (all medium compositions are w/v) toasted nutrisoy, 2% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, and 0.5% NaCl, adjusted to pH 7 with HCl.[7] One milliliter of a 72-h culture was used to inoculate 10 mL of medium in a 50-mL Erlenmeyer flask, and the flask was incubated at 28 °C, 200 rpm, for 1 day. Pleuromutilin or mutilin (5 mg in 0.1 mL of methanol) was added, and the incubation was continued for 2−5 days. The broth was extracted with 20 mL of ethyl acetate, and the extract was analyzed for pleuromutilin or mutilin. Samples in which pleuromutilin or mutilin was strongly depleted were analyzed by LC/MS to look for biotransformation products where the molecular weight had increased by 16, consistent with hydroxylation.

Preparative Batches for Hydroxylation of Mutilin by *S. griseus* SC 1754, *S. griseus* SC 13971, and *C. echinulata* SC 16162 and pleuromutilin by *C. echinulata* SC 16162. A 1-mL culture from a frozen vial was used to inoculate 100 mL of medium A in a 500-mL Erlenmeyer flask and the flask was incubated at 28 °C, 200 rpm, for 3 days. Fifty milliliters of this culture was used to inoculate 500 mL of medium A in a 4-L Erlenmeyer flask, and the flask was incubated at 28 °C, 200 rpm. Samples (5 mL) of the broth were extracted with 10 mL of ethyl acetate, and the extracts were assayed for pleuromutilin or mutilin by HPLC. Pleuromutilin was depleted to near zero after 6 days by *C. echinulata* SC 16162; mutilin was near zero after 2 days for *S. griseus* SC 1754 and 3 days for *S. griseus* SC 13971 and *C. echinulata* SC 16162. When pleuromutilin or mutilin was no longer detectable, the culture broth was extracted twice with 500 mL of ethyl acetate and once with 200 mL of ethyl acetate. Each extract was washed with 50 mL of saturated sodium chloride solution, and then the combined extracts were concentrated to about 40 mL at 40 °C under reduced pressure with a rotary evaporator. The concentrated extracts were evaporated to dryness under a nitrogen stream and used for isolation of products.

Pilot-Plant Batches for Hydroxylation of Mutilin by *Streptomyces* sp. SC 1754. A 1-mL culture from a frozen vial was used to inoculate 100 mL of medium A in a 500-mL Erlenmeyer flask, and the flask was incubated at 28 °C, 200 rpm, for 3 days. A second stage was started using a 1% inoculum from the first-stage flask and again incubated at 28 °C, 200 rpm for 3 days on medium A. A 5 or 10% inoculum was used to inoculate 15−100 L of medium B in a 15- or 100-L Braun fermentor and incubated at 28 °C, 400 rpm agitation, 1 vvm (volume/volume/min) air, 10 psig (0.689 bar gauge pressure), for 1 day. Medium B contained 2.0% toasted nutrisoy, 0.5% glucose, 0.5% yeast extract, 0.5% K_2HPO_4, and 0.1% SAG antifoam. The medium was adjusted to pH 7 with KH_2PO_4. Mutilin (50 mg/mL in methanol, 1% of batch volume) was added 24 h after inoculation to give a final concentration of 0.5 mg/mL. One hundred milliliter samples were taken from the fermentor after inoculation and again after mutilin addition and incubated in 500-mL flasks at 28 °C, 200 rpm, for comparison with the fermentor. Ten milliliter samples of the broth

were extracted with 20 mL of ethyl acetate every 24 h, and the extracts were assayed for mutilin and hydroxylated products by HPLC. The incubation was continued until mutilin was depleted, and 7- and 8-hydroxymutilin concentrations were maximal. The broth was extracted with 2 volumes of ethyl acetate, and the solvent was removed under vacuum at <40 °C.

HPLC Analysis. Ethyl acetate extracts were evaporated and dissolved in methanol. Samples were analyzed with a Whatman ODS-3, Partisil 5, 25 cm cm × 0.46 cm column at ambient temperature. The mobile phase was A (0.05 M KH_2PO_4 in water) and B (acetonitrile), with a gradient of 0 to 60% B over 120 min; flow rate was 1 mL/min, and detection was by UV absorption at 205 nm. Retention times were (1) 7-hydroxymutilin, 36 min, (2) 8-hydroxymutilin, 41 min, (3) 2-hydroxymutilin, 54 min, and (4) mutilin, 75 min.

Purification of Extracts from *S. griseus* SC 13971 and SC 1754 Biotransformation of Mutilin. The extract (292 mg) from biotransformation of mutilin by strain SC 13971 was subjected to silica gel column chromatography (14 g, 70−230 mesh silica gel, 1.5 cm × 20 cm). The column was eluted with a linear gradient (700 mL) of chloroform to 3% methanol in chloroform collecting 20 35-mL fractions. Fractions were monitored by TLC (9 μL spots, Analtech Uniplate GHLF, 10 cm × 20 cm silica gel plates, 40% acetone in hexane eluant, 2% vanillin in ethanol/ 0.1% sulfuric acid spray, heat). After TLC, fractions 11−20 were pooled and concentrated to give 181 mg of residue. This was subjected to silica gel column chromatography (14 g). The column was eluted with a linear gradient (700 mL) of hexane to 27% acetone in hexane, isocratically with 100 mL of 27% acetone in hexane and isocratically with 200 mL of 50% acetone in hexane collecting 28 35-mL fractions. Fraction 17 gave 8 mg of (2*S*)-hydroxymutilin. Fractions 19−20 were pooled and concentrated to give 89 mg of (8*S*)-hydroxymutilin. Fractions 23−25 were pooled and concentrated to give 19 mg of (7*S*)-hydroxymutilin. Fractions 21−22 were pooled and concentrated to give 27 mg of residue. This was refined further by silica gel column chromatography (14 g). The column was eluted with a linear gradient (700 mL) hexane to 60% ethyl acetate in hexane, then isocratically with 245 mL of 60% ethyl acetate in hexane collecting 27 35-mL fractions. Fractions 18 and 19 yielded additional (8*S*)-hydroxymutilin (5 mg), and fractions 21−24 yielded additional (7*S*)-hydroxymutilin (13 mg).

The extract (305 mg) from SC 1754 biotransformation of mutilin was processed exactly the same way as the extract from SC 13971. It yielded 16 mg of (2*S*)-hydroxymutilin, 100 mg of (8*S*)-hydroxymutilin, and 45 mg of (7*S*)-hydroxymutilin.

(2*S*)-Hydroxymutilin: ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, d, J = 7.10, H-17 Me), 0.94 (3H, d, J = 7.10, H-16 Me), 1.08 (1H, m, H-1a), 1.14 (3H, s, H-18 Me), 1.26 (3H, s, H-15 Me), 1.34 (1H, m, H-7a), 1.38 (2H, m, H-8), 1.60 (1H, d, J = 15.9, H-13a), 1.75 (1H, m, H-1b), 1.85 (1H, m, H-6), 1.85 (1H, m, H-13b), 2.07 (1H, m, H-10), 2.07 (1H, m, H-7b), 2.39 (1H, s, H-4), 3.36 (1H, d, J = 6.10, H-11), 4.04 (1H, m, H-2α), 4.27 (1H, d, J = 7.90, H-14), 5.26 (1H, dd, J = 11.1 and 1.1, H-20a), 5.33 (1H, dd, J = 17.8 and 1.1, H-20b), 6.10 (1H, dd, J = 17.8 and 11.1). ^{13}C NMR ($CDCl_3$): δ 10.7 (C-17), 13.6 (C-15), 18.1 (C-16), 27.1 (C-8), 28.6 (C-18), 32.6 (C-1), 34.4 (C-7), 35.6 (C-6), 36.0 (C-10), 41.4 (C-5), 44.1 (C-9), 45.0 (C-12), 45.1 (C-13), 56.4 (C-4), 66.7 (C-14), 70.5 (C-2), 75.4 (C-11), 116.0 (C-20), 139.2 (C-19), 216.1 (C-3). ^{1}H NMR NOE: 4.04 (H-2) to 2.07 (H7b).

(7*S*)-Hydroxymutilin: ^{1}H NMR (DMSO-d_6/$CDCl_3$): δ 0.68 (3H,d, J = 7.0, H-17 Me), 0.70 (1H, m, H-2a), 0.83 (3H, s, H-16 Me), 0.84 (3H, d, J = 7.1, H-18 Me), 1.11 (3H, s, H-15 Me), 1.20 (1H, m, H-6), 1.21 (1H, m, H-1a), 1.34 (1H, m, H-13a), 1.36 (1H, m, H-1b), 1.61 (1H, dd, J = 17.9 and 6.9, H-13b), 1.77 (1H, m, H-10), 1.78 (1H, m, H-2b), 1.81 (1H, m, H-4), 1.90 (1H, m, H-8a), 1.93 (1H, m, H-8b), 3.10 (1H, s, H-11), 3.32 (1H, m, H-7), 3.90 (1H, bd, J = 6.8, H-14), 4.92 (1H, d, J = 11.2, H-20a), 5.05 (1H, d, J = 17.9, H-20b), 5.85 (1H, dd, J = 17.9 and 11.2, H-19). ^{13}C NMR (DMSO-d_6/$CDCl_3$): δ 11.1 (C-17), 12.9 (C-16), 13.2 (C-15), 24.5 (C-1), 28.8 (C-18), 33.9 (C-8), 37.6 (C-10), 39.3 (C-2), 42.9 (C-12), 44.0 (C-6), 44.6 (C-5), 44.7 (C-13), 45.2 (C-9), 58.2 (C-4), 67.0 (C-14), 68.3 (C-7), 73.6 (C-11), 114.2 (C-20), 139.9 (C-19), 217.2 (C-3).

(8*S*)-Hydroxymutilin: ^{1}H NMR (DMSO-d_6): δ 0.77 (3H, d, J = 7.0, H-17 Me), 0.88 (3H, d, J = 7.3, H-16 Me), 0.99 (3H, s, H-18 Me), 1.26 (3H, s, H-15 Me), 1.29 (1H, m, H-7a), 1.48 (1H, m, H-13a), 1.50 (1H, m, H-1a), 1.60 (1H, m, H-7b), 1.63 (1H, m, H-1b), 1.76 (1H, m, H-10), 1.85 (1H, dd, J = 15.5 and 15.6, H-13b), 1.98 (4H, m, H-2a, H2b, H4, and H6), 3.35 (1H, m, H-11), 3.75 (1H, s, H-8), 4.04 (1H, bd, J = 4.4, H-14), 5.07 (1H, d, J = 11.3, H-20a), 5.28 (1H, d, J = 18.3, H-20b), 6.03 (1H, dd, J = 18.3 and 11.3, H-19). ^{13}C NMR (DMSO-d_6): δ 11.9 (C17), 13.7 (C-15), 18.0 (C-16), 23.2 (C-1), 30.0 (C-2), 30.0 (C-18), 35.4 (C-6), 36.3 (C-7), 39.1 (C-10), 41.2 (C-5), 44.9 (C-12), 45.6 (C-13), 49.4 (C-9), 54.9 (C-4), 65.0 (C-14), 70.6 (C-8), 73.2 (C-11), 113.9 (C-20), 141.8 (C-19), 215.8 (C-3).

Purification of (2*S*)-Hydroxymutilin from *C. echinulata* SC 16162 Biotransformation of Mutilin. The extract (305 mg) from *C. echinulata* SC 16162 biotransformation of mutilin was subjected to silica gel column chromatography (14 g, 70−230 mesh silica gel, 1.5 cm × 20 cm). The column was eluted with a linear gradient (700 mL) of chloroform to 3% methanol in chloroform collecting 20 35-mL fractions. Fractions 9−13 were pooled and concentrated to give 195 mg of residue. This was subjected to column chromatography (14 g of silica gel). The column was eluted with a linear gradient (700 mL) of hexane to 37% acetone in hexane collecting 20 35-mL fractions. Fractions 13−15 were pooled and concentrated to give 189 mg of residue. This was further refined by column chromatography (14 g of silica gel) using a linear gradient of chloroform to 50% ethyl acetate in chloroform and collecting 20 35-mL fractions. Fractions 10−16 were pooled and concentrated to give pure (by TLC and NMR) (2*S*)-hydroxymutilin (180 mg).

Purification of (2*R*)-Hydroxypleuromutilin from *C. echinulata* SC 16162 Biotransformation of Pleuromutilin.

The extract (234 mg) from *C. echinulata* SC 16162 biotransformation of pleuromutilin was subjected to silica gel column chromatography (14 g, 70−230 mesh silica gel, 1.5 cm × 20 cm). The column was eluted with a linear gradient (700 mL) of chloroform to 3% methanol in chloroform, collecting 20 35-mL fractions. Fractions 10−14 were pooled and concentrated to give 188 mg of residue. This was subjected to column chromatography (14 g silica gel). The column was eluted with a linear gradient (700 mL) of hexane to 37% acetone in hexane collecting 20 35-mL fractions. Fractions 14−18 were pooled and concentrated to give pure (2*R*)-hydroxypleuromutilin (173 mg).

(2*R*)-Hydroxypleuromutilin: ^{1}H NMR ($CDCl_3$): δ 0.68 (3H, d, J = 7.10, H-16 Me), 0.84 (3H, d, J = 7.00, H-17 Me), 1.16 (3H, s, H-18 Me), 1.18 (1H, m, H-8a), 1.30 (1H, m, H-13a), 1.31 (1H, m, H-1a), 1.32 (1H, m, H-7a), 1.40 (3H, s, H-15 Me), 1.44 (1H, m, H-7b), 1.48 (1H, d, J = 10.7, 11-OH), 1.80 (1H, m, H-8b), 1.86 (1H, m, H-6), 2.03 (1H, dd, J = 17.1 and 9.0, H-13b), 2.14 (1H, m, H-1b), 2.26 (1H, m, H-10), 2.36 (1H, t, J = 5.3, 2′-OH), 2.44 (1H, bs, H-4), 2.75 (1H, s, 2-OH), 3.32 (1H, dd, J = 10.7 and 6.4, H-11), 4.02 (2H, dd, J = 9.9 and 5.3, H-22), 4.08 (1H, m, H-2), 5.20 (1H, d, J = 17.4, H-20a), 5.34 (1H, d, J = 11.0, H-20b), 5.71 (1H, d, J = 9.0, H-14), 6.46 (1H, dd, J = 17.4 and 11.0, H-19). ^{13}C NMR ($CDCl_3$): δ 10.9 (C-17), 14.8 (C-15), 16.5 (C-16), 26.3 (C-18), 26.8 (C-7), 32.5 (C-8), 34.1 (C-1), 35.4 (C-6), 35.5 (C-10), 40.9 (C-5), 44.1 (C-9), 44.6 (C-12), 55.4 (C-4), 61.2 (C-22), 69.8 (C-14), 70.5 (C-2), 74.9 (C-11), 117.5 (C-20), 138.6 (C-19), 172.1 (C-21), 215.4 (C-3).

Purification of Pilot-Plant Extract from *S. griseus* SC 1754 Biotransformation of Mutilin. The dried extract (18.2 g) from a pilot-plant batch biotransformation of mutilin was dissolved in 100 mL of acetone and 50 mL of chloroform. Silica gel (45 g, 70−230 mesh silica gel) was added and the solvent removed in a rotary evaporator. The resulting powder was slurried in chloroform and added to a silica gel column (10 g, 70−230 mesh silica gel, 2.5 cm × 30 cm). The column was eluted with a step gradient of 1500 mL of chloroform, 1000 mL of 50% acetone in hexane, and 500 mL of 10% methanol in chloroform, collecting six 500-mL fractions. Fraction 4 yielded 7.2 g upon evaporation. This was purified further by column chromatography (45 g, 70−230 mesh silica gel). The column was eluted with a linear gradient (2000 mL) of chloroform to 5% methanol in chloroform, collecting 20 100-mL fractions. Fractions 5−17 were pooled and concentrated to give 5.0 g of residue. This was subjected to column chromatography (45 g, 70−230 mesh silica gel). The column was eluted first with a linear gradient (2000 mL) of hexane to 25% acetone in hexane and then with 25% acetone in hexane (1000 mL). Thirty 100-mL fractions were collected. Fractions 19−21 were pooled and concentrated to yield 920 mg of (8*S*)-hydroxymutilin. Fractions 23−26 were pooled to yield 230 mg of (7*S*)-hydroxymutilin.

Acknowledgment

We thank the following colleagues from Bristol-Myers Squibb: Susan Durand for HPLC analysis, John Venesky and Yanping Wang for LC/MS analysis of the biotransformation products, Yeung Chan, Victoria Browne, and Ken Bembenek for downstream processing of fermentation broths, and Dr. Qi Gao, Analytical Chemistry-Wallingford, for X-ray determinations of the structures of (7*S*)- and (8*S*)-hydroxymutilin.

Received for review February 18, 2002.

OP020028Q