

(+)-5'-HYDROXYGRISEOFULVIN

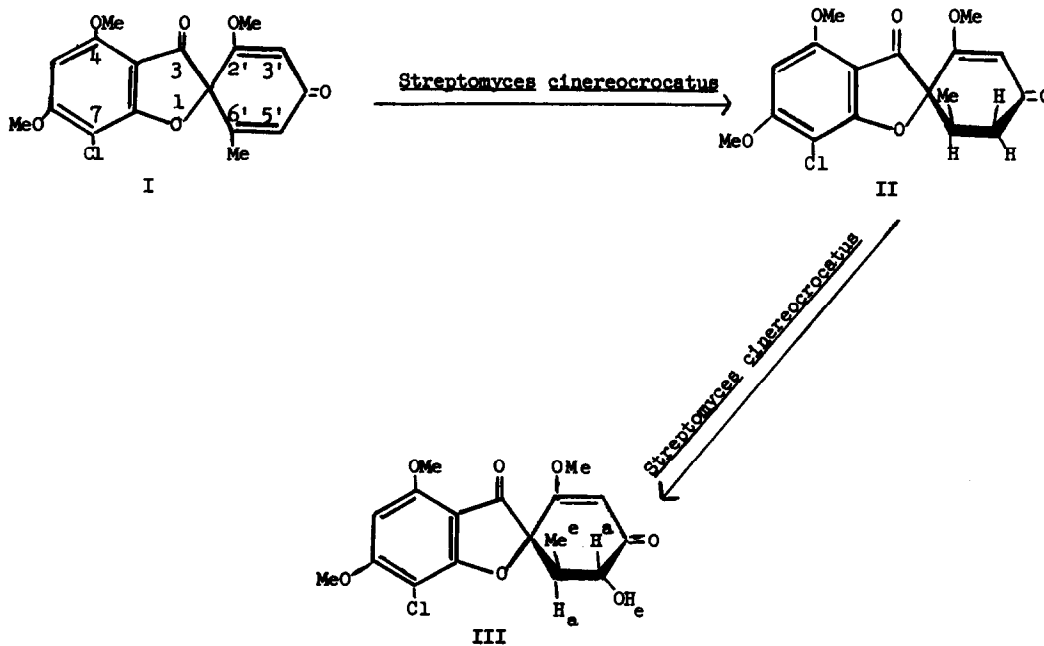
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We wish to report the preparation and characterization of the previously unknown (+)-5'-hydroxygriseofulvin. In our study of the preparation of pharmacologically useful compounds by microbiological transformation, it was observed that *Streptomyces cinereocrocutus* NRRL 3443 converted dehydrogriseofulvin (I)¹ into two products, one slightly less polar and the other more polar than the substrate. It was quickly established that the less polar product was (+)-griseofulvin (II). Thin-layer chromatographic time studies on fermentation concentrates indicated that the transformation was sequential in that the substrate was first converted to (+)-griseofulvin which in turn was converted to the more polar product. This latter product was isolated and shown to be the novel compound (+)-5'-hydroxygriseofulvin (III).



Crosse et al.² have studied the relationship between chemical structure and antifungal activity of griseofulvin derivatives. In the course of their work, they examined more than 300 analogues of (+)-griseofulvin with variations in substituents in the aromatic ring and at the 2' and 3' positions in the cyclohexenone ring. Synthesis of griseofulvin analogues continues to be an active field.³ A biologically active analogue of griseofulvin, lacking among other things a methyl group at the 6' position, has recently been reported.⁴ In so far as we are aware, our report is the first record of griseofulvin with modifications at the 5' position. (See Ref. 10).

Spores of Streptomyces cinereocrocutus NRRL 3443 were aseptically removed from a slant to inoculate 100 ml of sterile inoculum medium consisting of 1% soyflour x 200, 2% glucose, 0.5% cornsteep liquor and 0.3% calcium carbonate with pH unadjusted. After incubation for 72 hr at 25° on a rotary shaker (150 rpm) aliquots of 10 ml of inoculum were used to inoculate one-liter Erlenmeyer flasks containing 300 ml of sterile medium consisting of 3% cornstarch, 2% molasses, 1% soyflour x 200, 1% calcium carbonate and 0.25% yeast extract with pH unadjusted. After a 24-hr incubation period at 25° on a rotary shaker 30 mg of dehydrogriseofulvin or (+)-griseofulvin in 1 ml of acetone were added to each flask and incubation continued. At harvest time the pH was usually around 6.9. In scaled-up fermentations one-liter aliquots of two-stage inoculum were used to seed 30 liters of medium in stirred, aerated steel fermentors. In these fermentors 1.5 g of substrate were added in 50 ml of acetone. Partition thin-layer chromatography on cellulose (MM-polygram CEL 300 F254 - Brinkmann Instruments Inc.) was used to follow the progress of the transformation. Water was the stationary phase and the system hexane:ethyl acetate (4:1) was used to develop the chromatograms. The griseofulvin compounds were detected under black UV light and the R_f values of (+)-griseofulvin, dehydrogriseofulvin and (+)-5'-hydroxygriseofulvin were 0.57, 0.51 and 0.37, respectively.

The clarified harvest mash from a 30-liter fermentor was extracted with two one-third volumes of chloroform. The chloroform extracts were concentrated and the resultant solid subjected to partition chromatography over acid-washed diatomaceous earth using the system hexane:ethyl acetate:methanol:water (70:30:15:6). The solid from the sixth and seventh holdback volumes (20% yield) upon recrystallization from acetone-hexane had mp 222-224°, $[\alpha]_D^{25} + 292^\circ \pm 3$ (c 1, chloroform). Anal. calc. for C₁₇H₁₇O₇Cl: C, 55.50; H, 4.62; Cl, 9.66. Found: C, 55.08; H, 4.81; Cl, 9.15, m/e 368. The 60-MHz nmr spectrum of the material taken in CDCl₃ was most revealing especially in comparison with that of (+)-griseofulvin.^{5,6} In the latter case a complicated signal centered at 2.7 δ integrating for 3 protons is attributed to the 5'-methylene protons and the 6'-methine

proton. In the spectrum of the new griseofulvin analogue the complicated multiplet around 2.5 δ integrates for one proton only, and is due to the unchanged 6'-methine proton. A new signal, that of a one-proton doublet centered at 4.66 δ ($J = 12$ Hz) is due to the remaining 5'-proton. The downfield shift is accounted for by the presence of an electronegative hydroxyl group on the same carbon. The coupling constant $J = 12$ Hz between the 5'- and 6'-protons indicates a trans-diaxial arrangement⁷ for these protons thus placing the 5'-hydroxy group and the 6'-methyl group in equatorial positions.

Using acetic anhydride and pyridine, the acetate of the new analogue of griseofulvin was prepared, mp 254-256 $^{\circ}$, $[\alpha]_D^{25} = 297^{\circ} \pm 0.5$ (c 0.45, chloroform). Anal. calc. for $C_{19}H_{19}O_8Cl$: C, 55.54; H, 4.68; Cl, 8.62. Found: C, 55.53; H, 4.76; Cl, 8.30. The nmr spectrum of the acetate taken in $CDCl_3$ is similar to that of (+)-5'-hydroxygriseofulvin except for the singlet at 2.18 δ due to the methyl group of the acetate and the remaining 5'-proton doublet is shifted downfield ($\Delta\delta = 1.2$) and centered at 5.93 δ which is a characteristic shift for a proton alpha to an acetylated secondary alcohol.⁸

The methyl ether of the (+)-5'-hydroxygriseofulvin was also prepared by the method of Garden and Thompson⁹ and the product separated from unreacted material by partition chromatography. The methyl ether yield was 80%, mp 266 $^{\circ}$ $[\alpha]_D^{25} + 388^{\circ} \pm 2$ (c 1.0, chloroform). Anal. calc. for $C_{18}H_{19}O_7Cl$: C, 56.67; H, 4.96; Cl, 9.28. Found: C, 56.66; H, 5.07; Cl, 9.32. The nmr spectrum of the methyl ether derivative in $CDCl_3$ is similar to that of (+)-5'-hydroxygriseofulvin except that the multiplet for the 6'-proton is smeared out so broadly as to be almost undetectable and a sharp singlet at 3.58 δ now integrates for 6 protons due to the presence of the 2'- and 5'-methoxy groups. The chemical shift for the 5'-methoxy group is further evidence that it must be in the equatorial position since only in this position could it be in the deshielding cone of the carbonyl group.

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