NOTES

MICROBIAL TRANSFORMATION OF GRISEOFULVIN BY *CEPHALOSPORIUM CURTIPES*

P. Bod, E. Szarka,
J. Gyimesi, Gy. Horváth,
Zsuzsa Vajna-Méhesfalvi and I. Horváth

Research Institute for
Pharmaceutical Chemistry,
Fermentation Division,
Budapest, Hungary

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Boothroyd *et al.*[1], observed that as in higher organisms[2], certain fungi are capable of inactivating griseofulvin by demethylation. In addition, Andres *et al.*[3] reported a hydroxy derivative of griseofulvin, (+)-5′-hydroxygriseofulvin produced by *Streptomyces cinereocrocatus* NRRL 3443.

We have studied the microbial transformation of griseofulvin (I) by *Cephalosporium curtipes* and the formation of two more polar metabolites was observed in a proportion of 10 : 1. The compounds have been identified as (+)-5′-hydroxygriseofulvin (axial) (II) and 6′-hydroxymethylgriseofulvin (III) (Fig. 1).

The microbial transformation of griseofulvin was performed by a 48-hour-old culture of *Cephalosporium curtipes* strain. Cultivation was maintained on a rotary shaker (220 r.p.m.) at 24°C in a medium having the following composition: 1.5 % cornsteep liquor; 0.5 % soy bean meal; 0.5 % NaH_2PO_4 and 2.0 % glucose.

Twenty mg/liter griseofulvin was added to the culture at the 48 th, 60 th and 72 nd hours. The biooxidation process was followed by thin-layer chromatography and as a rule was stopped at the 120 th hour.

After filtration the fermentation liquid was extracted repeatedly with dichloroethane and the mycelium with acetone. The combined extracts were evaporated and the resultant solid was triturated with ethyl ether. The amorphous material obtained was a mixture of I, II and III. This was chromatographed on a silica gel column (300~400 mesh), using dichloroethane and ethyl acetate as developing solvents, the latter in increasing quantity (up to 60 %). Repeated separation (preparative thin-layer chromatography, Kieselgel G, Merck) and ethyl acetate – dichloroethane (1 : 1) yielded pure II and III after recrystallization from acetone.

Compound II; colorless crystalline solid m. p. 224~227°C. $[\alpha]_D^{25}$: +27.0±1° (*c* 0.5 chloroform). The UV spectra of I and II are identical. The IR spectrum of II is similar to I, but it has a sharp -OH band at 3480 cm^{-1}, m/e=368. The 60 MHz NMR spectrum of II (Fig. 2) (in $CDCl_3$): three $-OCH_3$ protons at 3.65, 4.0 and 4.05 ppm (δ), the olefinic and aromatic protons at 5.6 and 6.18 and a methyl doublet at 1.25 (with J=6 Hz coupling constant). On the basis

Fig. 1.

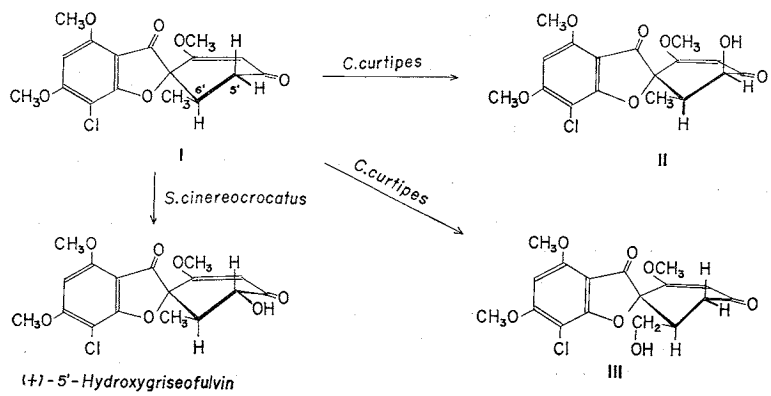


Fig. 2.

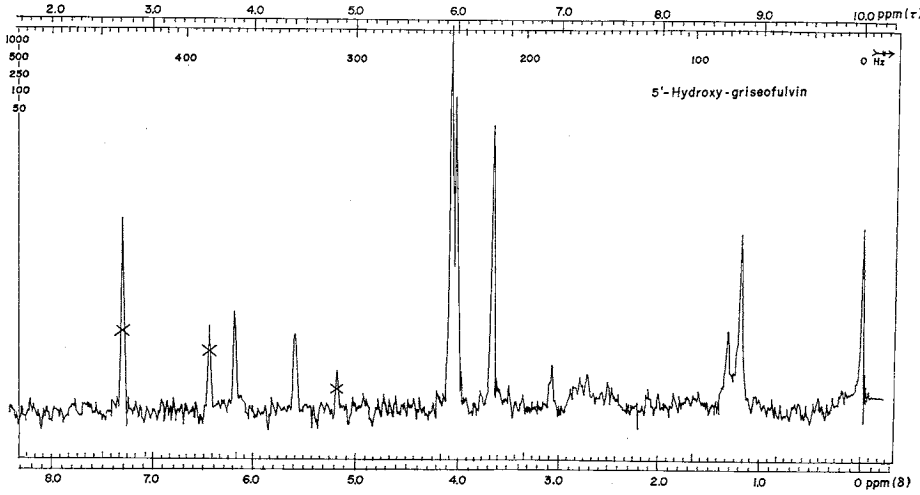
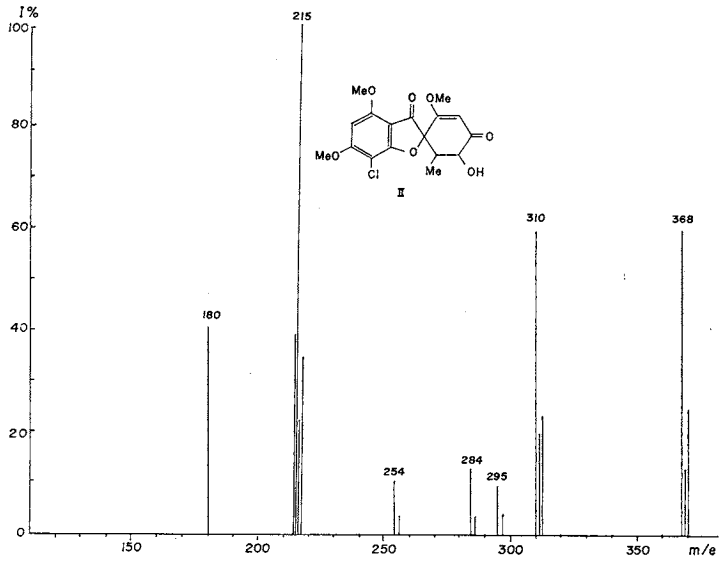


Fig. 3



of the methyl doublet, **II** is a 5'-hydroxy derivative.

A 5'-hydroxy derivative (equatorial) of griseofulvin is described in the literature, as mentioned above[8]. Our compound however, differs from this in several respects. The melting points differ slightly. The NMR spectra are not the same and the specific optical rotations differ considerably ($+27\pm1°$ and $+292\pm3°$, respectively). As would be expected for equatorial (+)-5'-hydroxygriseofulvin, (**II**) does not form an acetyl derivative in a mixture of pyridine-acetic anhydride.

Finally, in the mass spectrum of the deuterated derivative of **II**, the intensity of *m/e* 216 (Fig. 5) increases significantly and a new sign appears at *m/e* 181 (216-Cl), (Figs. 3 and 4).

Since the migration of the OH hydrogen takes place prior to the split of the carbon skeleton under the circumstances of mass spectroscopy (this can be explained by an intramolecular migration of the hydroxyl hydrogen to the keto group of the coumaranone ring. This reaction can take place only in

Fig. 4.

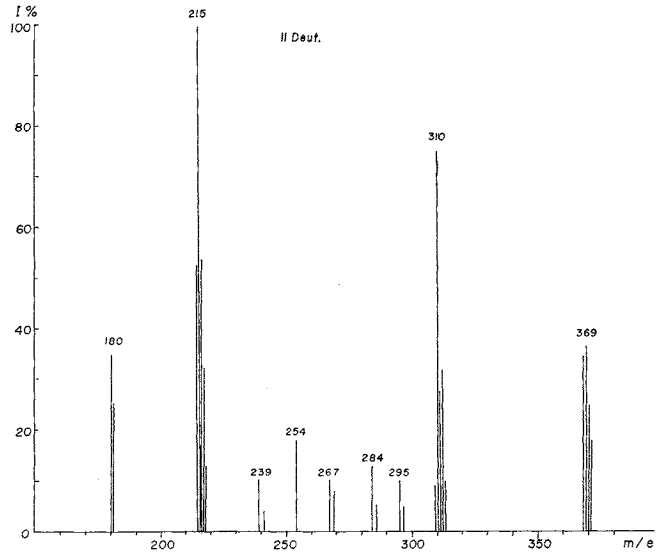
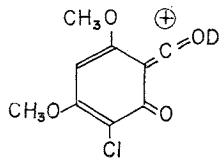
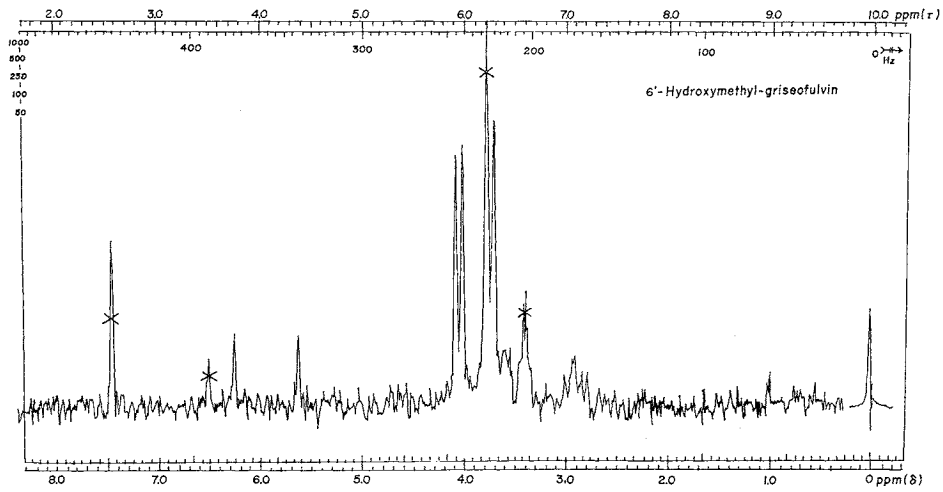


Fig. 5.



NMR spectrum of **III** (Fig. 6) the three $-OCH_3$ protons can be found at 3.7, 4.02 and 4.08. The olefinic and aromatic protons appear at 5.62 and 6.25. New signals appear at 2.95 and 3.60, the signal of the methyl group however, is not to be found. The methyl signal is missing in the IR spectrum. According to the above, **III** is (+)-6′-hydroxymethyl-griseofulvin.

Fig. 6.



the case of an axial 5′-hydroxyl group.

The evidence mentioned above shows that formula **II** (+)-5′-hydroxygriseofulvin (axial) represents the structure of the first hydroxylation product.

Compound **III**, colorless, solid, m.p. 213～216°C. UV spectrum in methanol is the same as **I** and **II**. The IR spectrum is griseofulvin-like too, but it has sharp-OH bands at 3520 and 3480 cm^{-1}, $m/e=368$. This compound is also a monohydroxy derivative. In the

References

1) Boothroyd, B.; E. J. Napier & G. A. Somerfield: The demethylation of griseofulvin by fungi. Biochem. J. 80: 34～37, 1961
2) Barnes, M. J. & B. Boothroyd: The metabolism of griseofulvin in mammals. Biochem. J. 78: 41～43, 1961
3) Andres, W. W.; W. J. McGahren & M. P. Kunstmann: (+)-5′-Hydroxygriseofulvin. Tetrahedron Letters 1969-43: 3777～3780, 1969