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METABOLITES OF *ASPERGILLUS VERSICOLOR*: 6,8-DI-*O*-METHYLNIDURUFIN, GRISEOFULVIN, DECHLOROGRISEOFLUVIN, AND 3,8-DIHYDROXY-6-METHOXY-1-METHYLXANTHONE

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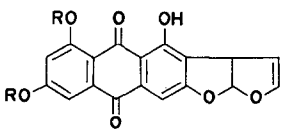
Key Word Index—*Aspergillus versicolor*; fungal toxins; dimethylnidurufin; dechlorogriseofulvin; biosynthesis.

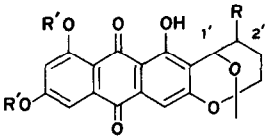
Abstract—The isolation of the four title compounds from cultures of *Aspergillus versicolor* is described and a modified biosynthetic pathway for the conversion of averufin to versicolorin A is proposed.

INTRODUCTION

The highly toxic and carcinogenic properties of the aflatoxins have fueled a growing concern that other microorganisms might be responsible for the generation of toxins and/or carcinogens on food and feed crops [1]. Among common food contaminants *Aspergillus versicolor* is of particular interest from this point of view since it is known to produce the anthraquinone versicolorin A (**1**), which has the same furanofuran ring system as the known toxins sterigmatocystin and aflatoxin B_1 and G_1 [2]. In addition, the known toxins luteoskyrin and rugulosin are dimeric hydroxyanthraquinones [3], and hence the possibility exists that some of the *A. versicolor* metabolites that do not possess the furanofuran ring system might be toxic or carcinogenic by virtue of their hydroxyanthraquinone system. Finally, recent observations that the *A. versicolor* metabolites averufin (**2**) [4] and versicolorin A [5] are intermediates in the biosynthesis of aflatoxin B_1 have increased the importance of investigating the occurrence of other potentially toxic metabolites in *A. versicolor*. In this paper we present the results of our initial investigations of the metabolites of *A. versicolor*, together with a proposed biosynthetic pathway for the conversion of averufin to versicolorin A.



(**1**) R = H
(**3**) R = Me



(**2**) R = H, R' = H
(**4**) R = OH, R' = H
(**5**) R = OH, R' = Me

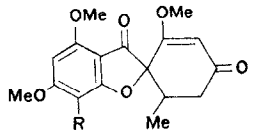
RESULTS AND DISCUSSION

A. versicolor (M 1004 or M 1214, obtained from A. F. Schindler, U.S. Food and Drug Adm., Washington, D.C.) was grown on autoclaved rice as described in the Experimental. Extraction of the rice cultured with M 1004 yielded a crude extract which gave a yellow crystalline material on purification. This material showed UV-visible absorption indicating the presence of a hydroxylated anthraquinone ring system similar to that in 6,8-di-*O*-methylversicolorin A (**3**) [6], while its IR spectrum showed absorptions indicating the presence of bonded and free quinone carbonyls [7]. Its NMR spectrum showed absorptions consistent with those expected for a dimethylated derivative of nidurufin (**4**) [8]. Thus a 3-proton singlet at 1.62 ppm is characteristic of the deshielded ketal methyl group and appears at about this position in nidurufin (1.58 ppm [8]) and tri-*O*-methylaverufin (1.64 ppm [9]), and the aromatic proton signals are consistent with those recorded for other compounds with this substitution pattern [2,6,8,9]. In addition, the 1-proton doublet at 5.28 is almost identical to that observed in nidurufin (5.17 ppm [8]), conclusively indicating the presence of only one proton on the 2′ carbon adjacent to the deshielded proton in the 1′ position.

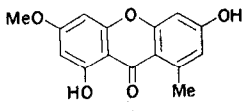
Confirmation of the nature of the 2′ substituent and of the side chain is provided by the mass spectrum of the new compound, which showed prominent peaks at *m/e* (M^+), 394 (M-18), 314 (M-98), and 99. The peaks at (M-18) and (M-98) occur as prominent peaks only in nidurufin and avermutin (averufanin) among various related anthraquinones [8], and only a dimethylnidurufin would have a molecular weight of 412.

The methoxy groups are assigned to the 6- and 8-positions on the basis of the chemical shifts observed for the aromatic protons on acetylation of dimethylnidurufin. The proton in the 4-position showed an acylation shift of almost 0.4 ppm (from 7.28 to 7.64 ppm) while the protons in the 5- and 7-positions showed essentially unchanged chemical shifts. This evidence confirms the presence of a free hydroxyl group in the 1-position and the absence of such a group in the 6- and 8-positions, and enables us to identify the new compound as 6,8-di-*O*-methylnidurufin (**5**).

The compounds isolated from the M 1214 strain of *A. versicolor* were identified as griseofulvin (**6**), dechlorogriseofulvin (**7**), and 3,8-dihydroxy-6-methoxy-1-methylxanthone (**8**) by their spectroscopic properties and melting points. This is the first time that these compounds have been isolated from an *Aspergillus* species, although all three have previously been obtained from various *Penicillium* species [10–12].

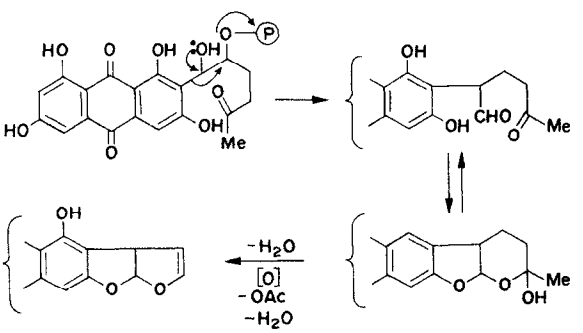


(**6**) R = Cl
(**7**) R = H



(**8**)

The isolation of a derivative of nidurufin serves to emphasize the importance of nidurufin in the metabolism of *A. versicolor*, and suggests that it may play a part in the biosynthesis of versicolorin A. It has already been shown that averufin (**2**) is converted by *A. parasiticus* into the aflatoxins B_1, B_2, and G_1 [4], and that versicolorin A (**1**) is also converted into the aflatoxins [5]. Although the direct conversion of averufin to versicolorin A has not yet been demonstrated, it has been widely assumed that this process does occur, and the conversion of a decaketide, of which averufin would be a possibility, into a nonaketide such as versicolorin A has been proposed [13]. An interesting feature of the putative pathway leading through versicolorin A is that the C-C bond joining the aromatic part of the molecule to the dihydrofuran ring is formed between two carbon atoms which *both* originate in the methyl groups (C-2) of acetate. Since averufin itself can readily be constructed from acetate units in the normal manner, it follows that the rearrangement which places two C-2 carbons of acetate adjacent to each other most likely occurs during the conversion of averufin to versicolorin A. At least two hypothetical mechanisms have been proposed for this rearrangement [13]; we wish to suggest a third, outlined in the



scheme below. In this scheme the key rearrangement step occurs through a pinacol-type rearrangement of the open-chain form of nidurufin. Subsequent steps involving cyclization, elimination of water, oxidative cleavage of a carbon-carbon double bond and recyclization, all have precedent in biochemical processes. While it is recognized that this mechanism is speculative, it does offer an alternative to the other mechanisms that have been proposed and it also has the advantage of involving a known metabolite of *A. versicolor* on the reaction pathway.

EXPERIMENTAL

Mp's are uncorr. IR spectra were as KBr discs and UV spectra were in EtOH. The NMR spectra were measured in $CDCl_3$ with TMS as internal standard. TLC was carried out on E. Merck Si gel GF-254 (analytical) or PF-254 (preparative).

6,8-Di-O-methylnidurufin (**5**). *Aspergillus versicolor* (strain M 1004) was cultured on autoclaved rice (4.3 kg) at 38° for 17 days. The pigmented mycelial mass, together with the rice, was extracted with $CHCl_3$–MeOH. The crude extract (4 g) was subjected to chromatography on a column of silicic acid (Mallinckrodt 100 mesh, 4 × 90 cm). Elution with $CHCl_3$ monitored by UV absorbance at 330 nm yielded 3 fractions: 1 (350 ml, first yellow band, 1.8 g), 2 (2400 ml, pale yellow eluate, 0.3 g), 3 (1300 ml $CHCl_3$ and then 600 ml MeOH, orange band, 1.7 g). Chromatography of fraction 3 on a column of Si gel (E. Merck) PF-254 (3 × 36 cm) and elution by EtOAc–hexane (1:4) with collection of 30 ml fractions yielded a yellow material (20 mg) in fractions 22–29. On recrystallization from Me_2CO–hexane it had mp 211–213°. The compound had the following physical properties $[\alpha]_D^{25} -77°$ ($CHCl_3$; *c* 0.15); MS:*m/e* (relative abundances) 412 (M^+, 64), 394 (32), 369 (12), 351 (18), 314 (100), 99 (42). UV: λ_{max} 224, 251, 288, 314 (inf.), 444 nm, ϵ 48,200, 19,000, 30,900, 8540, 8790. IR: ν_{max} 3500, 3420 (*br*), 2940, 1680, 1625, 1600, 1560, 1490, 1460, 1400, 1330, 1300, 1250, 1220, 1170, 1068, 1050, 1000, 970, 890, 850 cm^{-1}. NMR: δ 7.46 (1H, *d*, *J* 2.5 Hz), 7.28 (1H, *s*), 6.86 (1H, *d*, *J* 2.5 Hz), 5.30 (1H, *d*, *J* 2.0 Hz), 4.16 (1H, *m*), 4.04 (3H, *s*), 4.00 (3H, *s*), 2.5–1.6 (*c*, 4H, *m*), 1.64 (3H, *s*), 1.60 (1H, *s*).

Acetylation of dimethylnidurufin. Dimethylnidurufin (2 mg) was allowed to stand at room temp. for 5 days in C_5H_5N (0.5 ml) and Ac_2O (0.2 ml). Usual work-up gave a product showing 3 spots on TLC; purification of the crude product by preparative TLC (hexane–EtOAc, 3:7) gave one major orange product. The NMR spectrum of this product showed peaks at δ 7.64 (1H, *s*), 7.42 (1H, *d*, *J* 2.5 Hz), 6.84 (1H, *d*, *J* 2.5 Hz), 3.98 (6H, *s*), 2.66 (3H, *s*), 2.33 (3H, *s*), 1.68 (3H, *s*), 1.64 (*c*, 4H, *m*). MS: *m/e* (relative abundance) 496 (M^+, 10) 454 (15) 436 (5) 412 (70) 394 (100). IR: ν_{max} 1740 cm^{-1}.

Methylation of dimethylnidurufin. Dimethylnidurufin (3 mg) was converted to its methyl ethers with MeI–AgO. Purification of the crude product by preparative TLC (C_6H_6–EtOAc, 9:1, two developments) yielded trimethylnidurufin, mp 165–168° (lit. [8] 158–160°).

Griseofulvin, dechlorogriseofulvin, and xanthone (**8**). Extraction of the culture of *A. versicolor* strain M 1214 grown on rice (7.8 kg) with $CHCl_3$ yielded on oily extract (62 g) which was loaded on a column of Mallinckrodt silicic acid, 100 mesh (3.3 × 75 cm) and eluted with $CHCl_3$ to yield 4 fractions: 1 (450 ml, 9.3 g, yellow band) 2 (325 ml, 9.1 g), 3 (600 ml, 9.4 g, orange–brown) 4 (750 ml, MeOH elution, 30.1 g). Fractions 3 and 4 from this column were combined with the corresponding fractions from an extract of 3.2 kg rice to yield a total of 45 g of oily material. This was defatted by a hexane–aq. acetonitrile partitioning, and the crude acetonitrile soluble material (35 g) was loaded onto a column of Si gel 60 (5 × 80 cm). Elution with hexane–EtOAc or EtOAc–MeOH gave the following 7 fractions: 1 (500 ml, hexane–EtOAc 7:3, 0.75 g) 2 (500 ml, 50:50, 0.06 g) 3 (400 ml, 50:50, 1.0 g) 4 (600 ml, 50:50, 0.60 g) 5 (700 ml, 25:75, 0.50 g) 6 (1000 ml, EtOAc–MeOH, 9:1, 4.5 g) 7 (1900 ml, 50:50, 18 g). Crystallization of the major component of fraction 3 from Me_2CO yielded a material identified as 3,8-dihydroxy-6-methoxy-1-methylxanthone (**8**), mp 251–252° (lit. 253–255° [11]). Its spectroscopic properties were consistent with the assigned structure. Crystallization of the major component of fraction 6 from acetone yielded a material identified as dechlorogriseofulvin (**7**), mp 180–181° (lit. 179–181° [10]). The compound had $[\alpha]_D^{25}$ + 376° ($CHCl_3$, *c* 0.96) (lit. + 390° [10]), and its spectroscopic properties were consistent with the assigned structure. Purification of fraction 7 by a second Si gel column

(4 × 65 cm) and elution with hexane–EtOAc (2:3), with collection of 500 ml fractions, yielded a material identified as griseofulvin (**6**), mp 215–216° (lit. 220–221° [12]) on crystallization of the material eluted in fraction 5. The spectroscopic properties of the compound (IR, UV, MS) were consistent with the assigned structure.

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