A New Metabolite from *Aspergillus quadrilineatus*

Viqar Uddin Ahmad* and Viqar Sultana

H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-32/Pakistan

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From the mycelium of the fungus *Aspergillus quadrilineatus,* a new pigment 7-methoxyaverufin has been isolated besides mannitol, sterigmatocystin, versicolorin, ergosterol, and averufin.

Aspergillus quadrilineatus is a soil fungus belonging to the family Eurotiacea and the group *A. nidulans* [1]. Earlier workers have reported the isolation of quadrilineatin [2], sterigmatocystin [3] penicillin [4], the chlorine-containing compounds nidulin and nornidulin [4] as metabolites of this fungus. It has also been shown to produce the pigment asperthecin [5].

In the present study, *A. quadrilineatus* was grown on Czapek Dox medium [6] (pH 4–5). The mycelium was then extracted with ethanol, the residue obtained after removal of the solvent from the extract was extracted with chloroform. The chloroform insoluble residue was taken up in methanol, whereby a crystalline substance remained insoluble which was filtered recrystallised from methanol m.p. 161–165 °C. It was identified as mannitol (lit. m.p. 166 °C) through its IR, ^{1}H NMR, ^{13}C NMR and mass spectra, melting point of derivatives and direct comparison with an authentic sample. (Superimposable IR spectra, m.m.p.)

The chloroform residue was evaporated and the residue chromatographed on a column of silica gel. Hexane eluted pale yellow crystals m.p. 95–98 °C (lit. m.p. 95 °C) which were identified as elemental sulphur in its octameric form (M^+ 255.7760, calc. 255.7768). Other peaks were also identical to those given in the literature [7]. Sulphur is obviously produced by the fungus through the reduction of the sulphate ions present in the nutrient medium.

The hexane-chloroform mixture (1:1) eluted a compound which melted at 243 °C (lit. m.p. 246 °C) after crystallisation from acetone. The mass spectrum (M^+ at *m/z* 324 corresponding to the formula $C_{18}H_{12}O_6$) and the UV, IR, PMR spectra corresponded exactly to those reported for sterigmatocystin first isolated from *A. versicolor* [8].

* Reprint requests to Dr. V. U. Ahmad.

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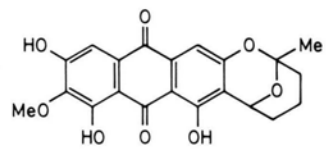
The fraction eluted with chloroform was a mixture of pigments and was rechromatographed on a column of silica gel. The first pure pigment which was eluted from this second column was recrystallised from acetone to yield yellow crystals m.p. 198–200 °C decomp. Its UV spectrum in methanol showed maxima at 207, 226, 290, 320, and 400 nm very similar in shape to that of averufin [9]. The infra-red spectrum (KBr) showed a hydroxy band at 3400 cm^{-1}, other peaks were present at 1670 (C=O), 1620 (hydrogen bonded C=O) and 1580 as generally seen in anthraquinone pigments. The mass spectrum showed a molecular ion peak at *m/z* 398.09851 (calc. for $C_{21}H_{18}O_8$, 398.1014). In addition strong peaks were present at *m/z* 380 (M^+-H_2O) 355, 340 (base peak) 327, 316, 312. The above spectral data indicated that the compound is a methoxy (or hydroxy methyl) derivative of averufin. The mass spectral peaks were indeed 30 mass units higher than those observed for averufin (see below), corresponding to the fragmentations of the acetal ring as suggested by P. Roffey; *et al.* in case of tri-o-methyl averufin [10].

The position of the methoxy group was determined to be 7 from the proton NMR spectrum of this new pigment. Whereas the spectrum ($CDCl_3$, 100 MHz) at higher field was similar to that reported [9] for averufin, it showed two singlets without any splitting at δ 7.1 and 7.2 due to H-4 and H-5, the H-7 signal which is present at δ 6.66 in averufin is missing here. The absence of the metacoupling also indicated that the two aromatic protons are in different rings. The spectrum also showed two singlets at δ 12.5 and 12.3 (phenolic protons) and a hump at δ 5.3 (H-1′). The methoxy singlet intergrating for 3 protons was present as expected at δ 4.10. The compound is therefore 7-methoxy averufin (**1**).



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The second substance eluted from the column was obtained, after crystallisation from methanol, as orange-red crystals m.p. 280–285 °C decomp. The mass spectrum of this compound showed molecular ion peak at *m/z* 368 along with strong peaks at 350 (M^+-H_2O) 325, 310 (base peak) 297 and 286. This compound was identified as averufin (lit. m.p. 280–282 °C decomp.), which was first isolated from *A. versicolor* [9], because its UV and NMR data

were identical to that reported for averufin. On acetylation at room temperature it yielded a diacetate m.p. 180–183 °C. The hydroxyl group which is hydrogen bonded to carbonyl group escapes acetylation under these conditions.

The third pigment isolated from the column was obtained after repeated crystallisation from methanol as yellow crystals m.p. 295 °C decomp. Its mass spectrum showed molecular ion peak at *m/z* 340.0593 (calculated for $C_{18}H_{12}O_7$ 340.0591) along with peaks at *m/z* 325 (M^+-CH_3) 310 (M^+-CH_2O) and 297 (base peaks M^+-CH_3-CO). This compound was identified as versicolorin B (lit. m.p. 298 °C decomp) [11], first isolated from *A. versicolor*, through the comparison of UV, IR and PMR data with those published in the literature [11].

The compound eluted with chloroform-hexane formed colourless crystals m.p. 164 °C. It gave positive test for sterols and in the mass spectrum it showed molecular ion peak at *m/z* 396 corresponding to the formula $C_{28}H_{44}O$. Other peaks were visible at *m/z* 378 (M^+-H_2O), 363, 337, 271 (M^+-side chain) 253, 211. This compound was identified as ergosterol (lit. m.p. 165 °C), ^1H NMR spectrum was completely, superimposable with the published [12] spectrum.

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