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DIHYDRO-O-METHYLSTERIGMATOCYSTIN, A NEW METABOLITE FROM ASPERGILLUS FLAVUS

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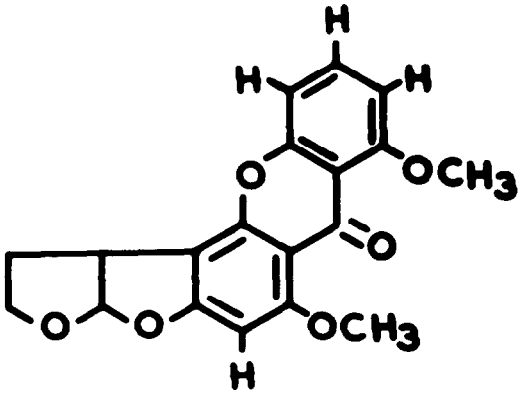
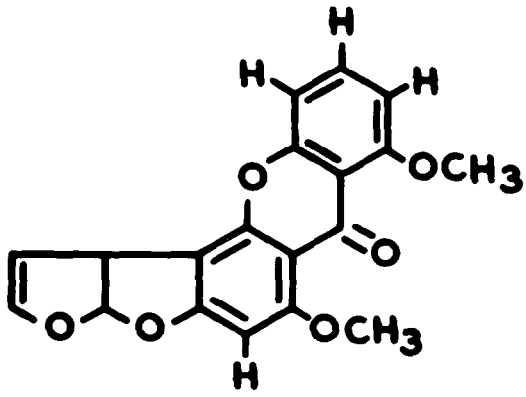
A new metabolite was isolated from a non-aflatoxin-producing strain of Aspergillus flavus. The metabolite was identified as a dihydro-derivative (I) of a previously-reported metabolite of A. flavus, O-Methylsterigmatocystin (II).[1] The structure was derived on the basis of melting point, thin-layer chromatography (TLC), infrared (I.R.), ultraviolet (U.V.), nuclear magnetic resonance (NMR), and mass spectral analyses. O-Methylsterigmatocystin (II) was also isolated from this mold and its identity confirmed by comparisons with authentic O-Methylsterigmatocystin. Although O-Methylsterigmatocystin has been previously found in a strain of A. flavus, as far as is known this is the first report of a sterigmatocystin derivative(s) isolated from a non-aflatoxin-producing strain of A. flavus.



I



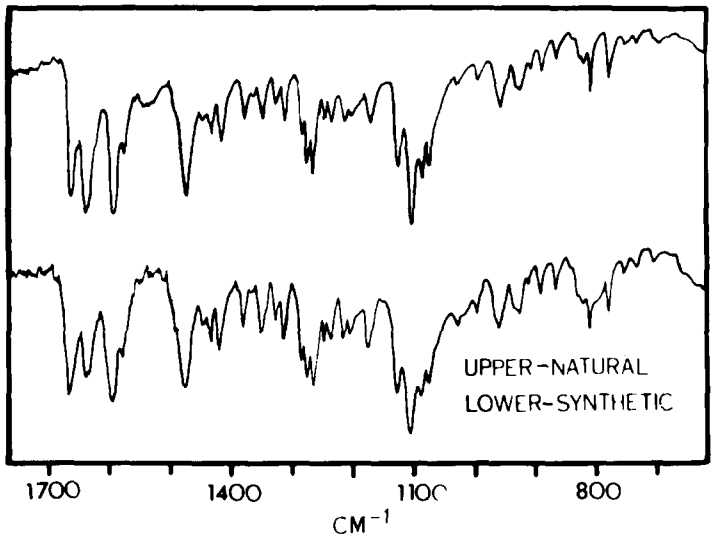
II

A. flavus was cultured in 500 ml erlenmeyer flasks containing 50 ml of Difco[2] mycological broth adjusted to pH 4.8 and supplemented with 20 g/liter yeast extract and 200 g/liter sucrose.

Cultures were incubated at 28 C for 5-7 days and subsequently extracted with 100 ml chloroform per flask in a Waring Blendor. The crude extract was chromatographed on columns (45 cm x 2 cm) containing Woelm[2] neutral grade II alumina using 10% n-hexane in chloroform as the eluting solvent and collecting 25 ml fractions. Fractions were monitored by Silica Gel GH-R TLC plates using chloroform-acetone (93:7 v/v) as the developing solvent. Two fluorescent compounds (R_f 0.32 and R_f 0.38) were detected in the column effluents by examining the TLC plates under long wave U.V. light. The fluorescence of these compounds was enhanced and changed from pale blue to bright yellow after spraying the TLC plates with 50% sulfuric acid.

Figure 1

Comparison of Infrared Spectra of Natural and Synthetic Dihydro-O-Methylsterigmatocystin



The fractions from alumina column chromatography containing these compounds were combined and re-chromatographed on columns containing Silica Gel again using 10% n-hexane in chloroform as the eluting solvent. The major component was eluted from the column before the minor component and it was obtained free of the minor component by combining these early fractions. The remaining combined fractions contained a 50:50 mixture of the two compounds. The minor component was obtained free of the major component after the third pass through the Silica Gel column. Both compounds were further purified by recrystallization from chloroform-methanol solutions. The ratio of the major compound to the minor compound was approximately 19:1.

The major compound was a colorless, crystalline material which melted (decomposed) at 265 C. The U.V. spectrum showed λ_{max}^{MeOH} 238 nm and 313 nm. Analysis of this compound by mass spectroscopy showed a base peak at 338 m/e and a molecular ion peak of 338. The NMR spectrum indicated that the compound contained 14 protons if it is assumed that the two singlets at δ3.92 and δ3.96 were due to methoxy groups. The carbonyl region of the I.R. spectrum indicated a γ-pyrone system. A search of the literature revealed that this compound was O-Methylsterigmatocystin.[1] This was confirmed by comparison of the U.V. and I.R. spectra and TLC characteristics of this compound with O-Methylsterigmatocystin obtained from Dr. H. J. Burkhardt, Western Regional Research Laboratory, Albany, California. The identity of the compound was further confirmed by comparison with melting point, NMR, and mass spectral data from published reports.[1,3]

The minor compound (R_f 0.32) had the same U.V. spectrum as O-Methylsterigmatocystin indicating that the chromophores were identical. Therefore, the most logical difference between these compounds would be in the dihydrodifurano portion of the molecule. A tetrahydrodifurano ring system was suggested by the lower R_f of this compound on TLC when compared with O-Methylsterigmatocystin (II). This is analagous to the R_f relationship of aflatoxin B_1 to B_2 and aflatoxin G_1 to G_2 on TLC.

The dihydro-derivative of O-Methylsterigmatocystin (I) was synthesized from purified O-Methylsterigmatocystin (II) by hydrogenation with 5% palladium catalyst on carbon in ethyl acetate at 25 C and atmospheric pressure.[3] The product was recovered in the usual manner, chromatographed on a column containing Silica Gel and further purified by recrystallization from chloroform-methanol solution. The purified product had a melting point of 281-282 C which compared favorably with the melting point reported in the literature (280 C) for dihydro-O-Methylsterigmatocystin[3] and also with the melting point determined for the minor compound isolated from A. flavus (280-282).

The U.V. spectrum for synthetic dihydro-O-Methylsterigmatocystin (I), natural dihydro-O-Methylsterigmatocystin, and O-Methylsterigmatocystin were identical (λ_{max}^{MeOH} 238 nm and 313 nm). The I.R. spectra of the natural compound and the synthetic compound were almost identical (Fig. 1). The only difference noted was the relative intensity of the 1640 cm^{-1} absorption and the tendency of this peak to be split in the spectrum of the synthetic compound.

The mass spectrum of the synthetic dihydro-derivative gave a base peak at m/e 340 and a molecular ion peak of 340. The mass spectrum of the A. flavus dihydro-derivative matched the spectrum of the synthetic compound peak for peak including a base peak at m/e 340 and a strong doubly charged peak at m/e 162.5 which was produced from fragments of a strong peak at m/e 325. Most of the fragment peaks over mass 200 were 2 mass units greater than the corresponding fragment peaks of O-Methylsterigmatocystin.

The NMR spectra of the natural and synthetic dihydro-derivatives were in complete agreement. The two additional protons on the tetrahydrodifurano ring system shifted the peaks well upfield and doubled the area of these peaks (δ3.65, 2 protons, multiplet and δ2.30, 2 protons, multiplet). The proton at the junction of the two furan rings also was shifted upfield (δ4.85, 1 proton, triplets of doublet shifted to δ4.20, 1 proton, multiplet).

Acknowledgment

We wish to thank Dr. S. R. Dutky, Insect Physiology Pioneering Res. Lab., Beltsville, Md., for performing the NMR and mass spectral analyses and with aid in the interpretation of these spectra. We also thank Dr. H. J. Burkhardt, Western Regional Res. Lab., Albany, California, for the sample of O-Methylsterigmatocystin.

Note Added in Proof: Gas-liquid chromatographic analyses of natural and synthetic dihydro-O-Methylsterigmatocystin with SE-30 liquid phase at a column temperature of 232 C showed the relative retention time (relative to cholestane) of the natural compound (1.85) was identical to the relative retention time of the synthetic compound (1.85). GLC analyses were performed by Dr. S. R. Dutky, Entomology Research Division, U. S. Department of Agriculture, Beltsville, Md.

References

1. H. J. Burkhardt and J. Forgacs, Tetrahedron, 24, 717 (1968).
2. Reference to a company or product name does not imply recommendation of the product by the U. S. Department of Agriculture.
3. J. E. Davies, D. Kirkaldy, and J. C. Roberts, J. Chem. Soc., 2169 (1960).