*Tetrahedron* Vol. 24, pp. 717 to 720. Pergamon Press 1968 Printed in Great Britain

# O-METHYLSTERIGMATOCYSTIN, A NEW METABOLITE FROM *ASPERGILLUS FLAVUS*, LINK EX FRIES

H. J. BURKHARDT and J. FORGACS*

Western Regional Research Laboratory,† Albany, California 94710

(*Received in USA* 29 *March* 1967; *accepted for publication* 19 *May* 1967)

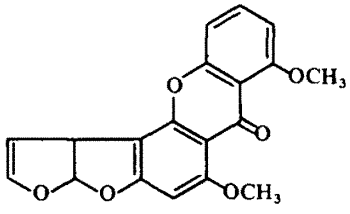
**Abstract**— A new metabolite has been isolated from the aflatoxin-containing fraction of *Aspergillus flavus* and identified as O-methylsterigmatocystin. This structure was derived from elemental analysis, NMR, IR, UV and mass spectral data and confirmed by identity with synthetic O-methylsterigmatocystin.

## INTRODUCTION

IN THE course of work on mycotoxicoses Forgacs and Carll[1] isolated several toxigenic fungi from cycad endosperm. They reported high toxicity in mice for one strain of *Aspergillus flavus*. The toxicity of this strain was higher than that arising from aflatoxin production in other strains of *A. flavus*. This report is concerned with the origin of the high toxicity of the strain isolated from cycad (strain I) compared to a strain of *A. flavus* (strain II) known to produce aflatoxin. It also describes the isolation and identification of a new fluorescent compound, only found in strain I.

## RESULTS

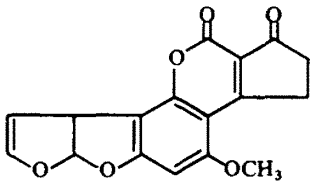
The aflatoxin fractions of strain I and II of *A. flavus* showed that strain I produced about 100–150 times as much aflatoxin as strain II. This was established by dilution of aliquots, their chromatography and visual comparison under ultraviolet light and by actual isolation of purified aflatoxin. It was also noted that strain I produced more aflatoxin G than B, while strain II produced more aflatoxin B than G. An additional yellow fluorescent component with a somewhat higher $R_F$ value than that of aflatoxin B was present only in strain I. This compound was separated by column chromatography and its structure determined as O-methylsterigmatocystin (formula I).



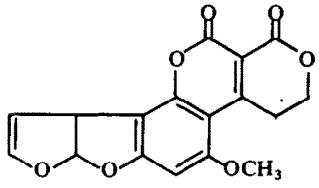

I

* Present address: Good Samaritan Hospital, Suffern, New York.

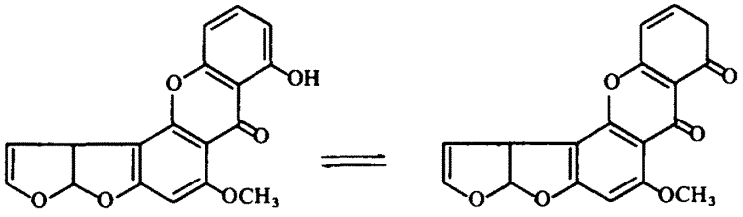
† A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.



II



III



IV

Elemental and mass spectral analysis enabled us to arrive at its empirical formula as $C_{19}H_{14}O_6$. This was confirmed by NMR spectroscopy where 14 protons were recorded. Two 3-proton singlets at $\delta$ 3·92 and $\delta$ 3·96 indicated the presence of two MeO groups. Additional signals for four protons at $\delta$ 6·82 (doublet $J = 7$ c/s), $\delta$ 6·50 (triplet, $J = 2{\cdot}5$ c/s), $\delta$ 5·46 (triplet, $J = 2{\cdot}5$ c/s), and $\delta$ 4·81 (triplets of doublet, $J = 2{\cdot}5$ and 7 c/s) revealed a striking similarity with the four protons of the dihydrodifurano ring system of aflatoxin B (II) and G (III).[2] The remaining four protons at $\delta$ 6·38 (singlet), $\delta$ 7·48 (triplet, $J = 8{\cdot}1$ c/s) and a group of two protons at $\delta$ 6·82 superimposed on one of the protons of the dihydrodifurano ring system ($J = 8{\cdot}1$ and 1·5 c/s) were assumed to be aromatic protons, 3 being adjacent to each other and one isolated from the others.

The IR spectrum with its C=O absorption at 1664 $cm^{-1}$ was indicative of a $\gamma$-pyrone system, and accounted for both the remaining oxygen atoms. The combination of all data led to the conclusion that the isolated compound might represent a dihydrodifurano substituted dibenzo-$\gamma$-pyrone-dimethyl ether. Literature study revealed the compound was known as O-methylsterigmatocystin (I) and had been synthesized from sterigmatocystin (IV).[3]*

* A 1-mg sample of this material was obtained through the courtesy of Dr. J. Roberts, University of Nottingham, England, and complete identity established by mixed m.p. and IR spectroscopy (Fig. 1).

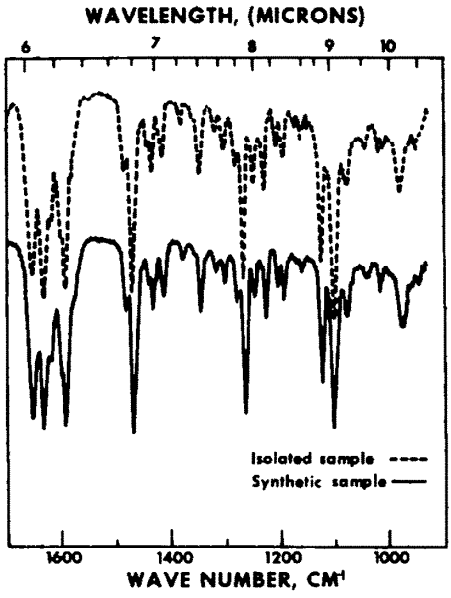



FIG. 1 Comparison of IR spectra of isolated and synthetic O-methylsterigmatocystin.

## DISCUSSION

The high toxicity of strain I, isolated from cycad rests to a great extent or wholly on the high production of aflatoxin. O-Methylsterigmatocystin which has not been found in strain II does not seem to contribute to the toxicity of strain I as indicated by preliminary biotests performed on mice and ducklings.

O-Methylsterigmatocystin (I) has structural similarity with aflatoxin $B_1$ (II) and $G_1$ (III) as far as the dihydrodifurano ring system is concerned but a possible diketo configuration in the remainder of the molecule is blocked by the dimethyl ether configuration. Sterigmatocystin (IV) itself has both these structural features and shows carcinogenicity but is not as toxic as aflatoxin $B_1$.[4] Sterigmatocystin has been isolated by three independent groups and found to occur in *Aspergillus versicolor*.[3] The final structure has been determined as IV by Bullock *et al.*[5] O-Methylsterigmatocystin and sterigmatocystin are dihydrodifuranoxanthones. Xanthones have been found in plants and fungi,[6] but sterigmatocystin and its derivatives are so far the only known dihydrodifurano derivatives of xanthones.

## EXPERIMENTAL

*Materials and methods*

*Physical.* UV absorption spectra were obtained with a Cary Model 14 recording spectrophotometer.[7] PMR spectra were recorded with the Varian A-60 high resolution NMR spectrometer. Mol. wts were determined with a Model 110-B (Consolidated Electrodynamics Corporation) mass spectrometer. IR spectra were obtained with a Beckman Model IR7 or Cary Model 90 spectrophotometer.

*Chromatography.* Aluminum oxide (active, neutral for chromatography, Merck) was used for preparative column chromatography and the procedure outlined by Neher[8] was utilized. TLC was performed on silica gel G (Merck) coated plates with detection of components by fluorescence (Mineralight Model SL 3660, Ultraviolet Products, Inc.).

*Strains of* A. flavus. Strain I (cycad strain): supplied by Dr. Joseph Forgacs, Good Samaritan Hospital, Suffern, New York. Strain II: supplied by U.S. Food and Drug Administration, Washington, D.C., No. 3734-10.

*Cultivation of* A. flavus. Both strains were grown in Fernbach flasks in 200 ml of the following medium/flask: water (1000 ml); $NaNO_3$ (2·0 g); ($K_2HPO_4$) 1·0 g; $MnSO_4$ (0·5 g); KCl (0·5 g); $FeSO_4$ (0·01 g) and agar (20 g). The flasks were incubated at room temp in the dark and harvested when sporulation was at its peak (14–23 days).

*Isolation of aflatoxin fraction (Strain I).* Each Fernbach flask was surface extracted with five 100-ml portions of $CHCl_3$, allowing 1 hr for each extraction. The combined $CHCl_3$ extracts of 10 flasks were filtered, dried over $Na_2SO_4$ and concentrated to a volume of 25 ml. To this was added 250 ml heptane and the ppt formed was separated, washed with pentane and dried over paraffin *in vacuo* yielding solid I (399 mg).

*Isolation of aflatoxin fraction (Strain II).* Isolation was as described for strain I but 20 flasks were harvested and the $CHCl_3$ extract concentrated to 5·0 ml and 100 ml heptane added. The yield was 8 mg. This material was purified by preparative TLC on one 20 × 20 cm × 0·5 mm plate and a total of 4 mg of aflatoxin ($B_1$, $B_2$, $G_1$ and $G_2$) obtained after elution of the corresponding zones with MeOH (solvent system: $CHCl_3$–MeOH = 97–3).

O-*Methylsterigmatocystin.* Solid I (399 mg) was dissolved in a mixture of $CHCl_3$ (2·0 ml) and acetone (20·0 ml) and adsorbed on 10 g of $Al_2O_3$ activity III. The $Al_2O_3$ was dried in the open air at room temp (vacuum at 60° destroys aflatoxin adsorbed on $Al_2O_3$) and trickled through heptane on top of a 34 × 250 mm column poured with $Al_2O_3$, activity III, in heptane. Elution was started with a mixture of heptane and $CHCl_3$ (7:3), which moved a yellow fluorescent zone (O-methylsterigmatocystin) relatively fast and also separated a slow moving blue fluorescent band (aflatoxin B) from a slow yellow fluorescent one (aflatoxin G). After elution of the sharp band representing O-methylsterigmatocystin the solvent was changed to $CHCl_3$–MeOH (19:1) to elute all the four aflatoxins in one fraction. The solvents of the two fluorescent fractions were evaporated and yielded 230 mg of aflatoxin ($B_1$, $B_2$, $G_1$, $G_2$) and 61 mg of O-methylsterigmatocystin, which was recrystallized from MeOH and from $CHCl_3$–heptane for elemental analysis and spectral data, m.p. 265° dec, $\lambda_{max}$ 236, 310 mμ (log ε, 4·614 and 4·224 respectively). (Found: C, 67·3; H, 4·35. Calc. for $C_{19}H_{14}O_6$: C, 67·45; H, 4·17%.)

*Acknowledgements*—The authors are indebted to Dr. J. C. Roberts for a sample of O-methylsterigmatocystin, and to Robert Maben for valuable assistance. Grateful thanks are expressed for obtaining the NMR, IR and mass spectral data to Drs. R. E. Lundin, J. Scherer and W. H. McFadden.

## REFERENCES

[1] J. Forgacs and W. T. Carll, *Fed. Proc.* **23**, 1370 (1964).
[2] T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Am. Chem. Soc.* **87**, 882 (1965).
[3] J. E. Davies, D. Kirkaldy and J. C. Roberts, *J. Chem. Soc.* 2169 (1960).
[4] J. Roberts, private communication (1965).
[5] E. Bullock, J. C. Roberts and J. G. Underwood, *J. Chem. Soc.* 4179 (1962).
[6] F. H. Dean, *Naturally Occurring Oxygen Ring Compounds* p. 226. Butterworths, London (1963).
[7] Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.
[8] R. Neher, *Steroid Chromatography* p. 10–33. Elsevier, New York (1964).