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3-HYDROXY-6,7-DIMETHOXYDIFUROXANTHONE - A NEW METABOLITE FROM ASPERGILLUS FLAVUS

A. C. Waiss, Jr., M. Wiley, D. R. Black, and R. E. Lundin

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

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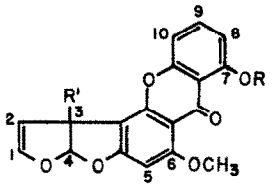
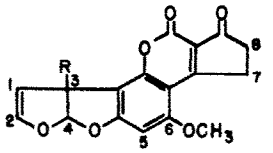
During the isolation of aflatoxin from cultures of Aspergillus flavus (1) a new metabolite was isolated. The structure of the compound was established as 3-hydroxy-6,7-dimethoxydifuroxanthone (Ia) by chemical and spectroscopic methods.



Ia R = CH_3, R' = OH

Ib R = CH_3, R' = H

Ic R = H, R' = H



IIa R = H

IIb R = OH

The new metabolite Ia (R_f = 0.65) (2) appears as a single blue fluorescent spot on thin-layer chromatographic plate migrating slightly ahead of aflatoxin M_1 (IIb, R_f = 0.60). Upon separation and purification on fine silica gel H columns, by gradient elution with $CHCl_3$ and 5%, MeOH-$CHCl_3$ mixture, Ia is obtained as white needles, m.p. 325-327°; $[\alpha]_D^{27}$-140° (C = 0.015, DMF) insoluble in most organic solvents, however, crystallizable from hot dimethylformamide. The compound analyses for $C_{19}H_{14}O_7$ (Calcd. C, 64.4; H, 3.98. Found: C, 64.1; H, 4.08) and gives a molecular ion peak by high resolution mass spectroscopy of m/e 354.0772, and exhibits λ_{max}^{MeOH} 309 (13,000) and 241 mμ (39,000) suggesting the possibility of a 6,7-dimethoxydifuroxanthone (O-methylsterigmatocystin), Ib nucleus (3). In support of this Ia gives a negative $FeCl_3$ test and its UV spectrum is unaffected by the addition of $AlCl_3$, evidence which eliminates the possibility of a O-hydroxybenzophenone structure. A negative Gibbs test (5) supports the same conclusion. The IR (KBr) spectrum of Ia, absorbing at 1660 (C=O) and 3280 cm^{-1}, (OH) is consistent with the proposed structure and further reveals

the presence of a hydroxy group (4). The NMR spectrum of Ia provides additional evidence for the details of the proposed structure. A comparison of the NMR spectra of 6,7-dimethoxydifuroxanthone (Fig. Ib) and the new metabolite Ia (Fig. Ia) clearly shows the similarity in the aromatic protons (H_5, H_8, H_9 and H_{10}) of the two compounds. With the cumulative diamagnetic effects from two ortho and a para - ethereal oxygen atoms, H_5 (3.44 τ) is expected to be a singlet absorbing at higher field than any other aromatic protons in the molecules. On the other hand, H_9 appears at lower field as a triplet at 2.39 τ ($J = 8H_z$) since it is ortho to H_8 and H_{10} and para to the deshielding carbonyl functions. The presence of two aromatic methoxyl groups is substantiated by a six proton singlet at 6.07 τ. Introduction of a hydroxyl group at C_3 expectedly transforms H_1 and H_2 (3.28 and 4.24 τ, respectively) into doublets, while H_4 (3.62 τ) collapses to a singlet. This transformation is completely analogous to the one between aflatoxin B_1 (IIa) and M_1 (IIb).

Since the discovery of the first difuro-mold metabolite, stigmatocystin, from Aspergillus versicolor in 1962 (6), numerous other natural and synthetic difuro-containing xanthones (3, 6, 7) anthraquinones (7, 8) and coumarins (9, 10) have been obtained. With the recent enhanced interest

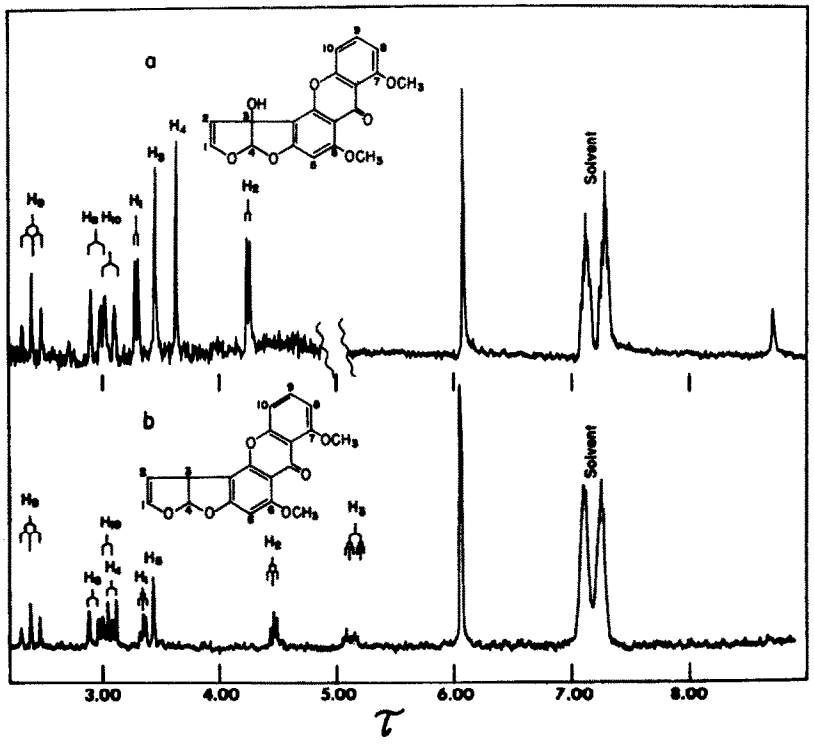
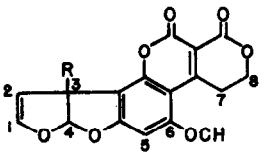


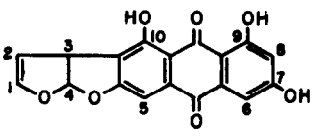
FIG. 1. 100 MH_z NMR spectra of (a) Ia and (b) Ib in DMF-d_6 at 90°.

in mold metabolites it is not unreasonable to expect that many more linear difuro derivatives will soon be discovered. The nomenclature of this class of compounds, however, has become increasingly complex (e.g., aflatoxin B_{2a} and G_{2a} (11), not to mention the indescribable synthetic derivatives) so that common names should now be introduced. Since the linear difuro-structure is the only common feature of these metabolites, any meaningful system of nomenclature should be based on this unit. Thus we suggest the name 3-hydroxy-6,8-dimethoxydifuroxanthone for Ia, 6-methoxydifurocoumarone (IIa) for aflatoxin B_1, 6-methoxydifurocoumarolactone (IIIa) for aflatoxin G_1, and 7,9,10-trihydroxy-difuroanthroquinone for versicolorin A (IV) (8), respectively.



IIIa R = H

IIIb R = OH



IV

The isolation of Ia from Aspergillus flavus culture is worth noting for at least two reasons: From a biogenetic point of view the co-occurrence of 6-methoxydifuroxanthone, 6-methoxydifurocoumarone, 6-methoxycoumarolactone, and some of their 3-hydroxy- and 1,2-dihydro-derivatives strongly suggests a common biosynthetic scheme for these compounds and that compounds such as IIIb and dihydro-difuroxanthones which are not yet discovered might be present also in the culture. Although chemotoxonomy has been widely applied in higher plants, the occurrence of difuroxanthone derivatives in both Aspergillus versicolor and flavus indicates that chemotoxonomy may be applicable also for lower organisms.

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