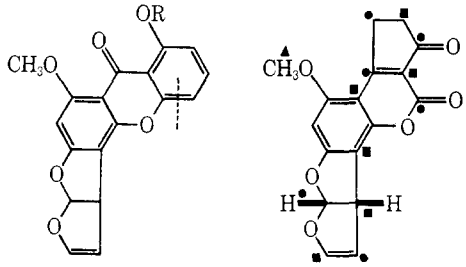
diradical **3** (or zwitterion) to the aldehyde **4**. A further isomerization, similar to one encountered in the *in vitro* synthesis of aflatoxin-B_1,[2] leads to versicolorin A (**5**, R = OH)[3] and to aversin (**6**, R = OCH_3).[4] The two metabolites **5** and **6** may also arise from the polyhydroxybenzanthracene **7** by an entirely analogous sequence leading to the same distribution of labels. The rearrangement of the *endo*-peroxide **2** to the pyran **4** seems to be without chemical precedent, yet it does provide an exceedingly economical and mechanistically not unreasonable pathway to the bisfuran moieties of metabolites elaborated by the genus *Aspergillus*.

It has previously been postulated[5] that the difuroxanthone sterigmatocystin (**8**)[6] is derived from an anthraquinone by oxidative ring cleavage (dotted lines in **5**), and experimental evidence in favor of such a cleavage has recently been secured[7] for the biosynthesis of ergochromes.

The structural similarity between sterigmatocystin (**8**) and aflatoxin-B_1 (**10**) as well as the coexistence of O-methylsterigmatocystin (**9**)[8] and aflatoxins in *A. flavus* has led to the postulate that a difuroxanthone is an intermediate in the biosynthesis of the aflatoxins. Two detailed schemes were presented,[5,9] but only one[5] involving oxidative ring cleavage (dotted line in **8**) and recyclization followed by expulsion of an acetate *methyl* derived carbon atom leads to the distribution of label in the cyclopentane moiety demanded by our experimental findings.

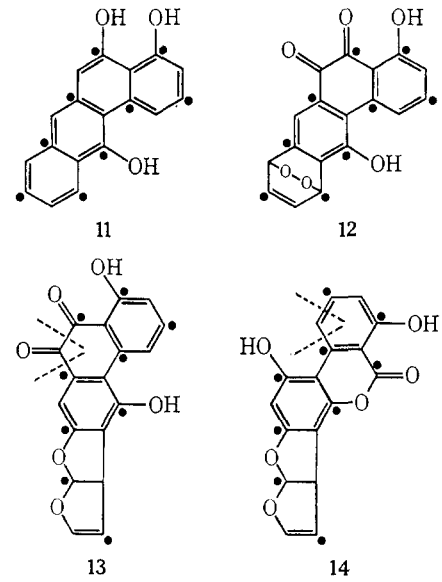


8, R = H
9, R = CH_3

10, CH_3COOH
$CH_3SCH_2CH_2CH(NH_2)COOH$

Since the only experimental evidence available[9] is against sterigmatocystin (**8**) being a precursor of aflatoxin-B_1 (**10**) in *A. flavus*, one should not overlook the possibility that the aflatoxins could originate from a trihydroxybenzanthracene (**11**) isomeric with **7** by the route **11** → **12** → **13** → **14** → **10**.

Finally, aflatoxin-M_1[10] and aspertoxin[11,12] (hydroxy-



11 12 13 14

O-methylsterigmatocystin) are almost certainly derived from aflatoxin-B_1 (**10**) and O-methylsterigmatocystin (**9**) rather than *vice versa* because the additional hydroxy group present in the bisfuran portion of these metabolites is attached to an acetate methyl group.

Acknowledgment. This study was supported by Contract No. PH 43-62-468 with the National Cancer Institute, National Institutes of Health.

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Incorporation of Precursors into Aflatoxin-B_1

Sir:

Previous studies[1] have implicated phenylalanine as a precursor of aflatoxin biosynthesis by cultures of *Aspergillus flavus*. When DL-[alanine-3-^{14}C]phenylalanine was added to a resting cell culture of *A. flavus* ATTC 15517 metabolizing glucose, the RIC[2] of the aflatoxin-B_1 extracted from the culture broth was 0.16. However, when DL-[ring-^{14}C]phenylalanine, L-[alanine-1-^{14}C]phenylalanine, or L-[gen-^{14}C]phenylalanine was added in similar concentrations, the RIC values were about 0.01–0.02.[3] These results suggested[4] that the efficient labeling observed initially[1] resulted from catabolism of the added phenylalanine by enzymes induced by the relatively high (0.5–1.0 m*M*) concentration of added phenylalanine. The necessity of using a high concentration of added phenylalanine to suppress synthesis of endogenous phenylalanine can be obviated by using a phenylalanine-requiring mutant. Such a mutant, *A. flavus* A77, was grown in the presence of 0.1 m*M* DL-[ring-^{14}C]phenylalanine. After a 7-day incuba-

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(2) RIC, relative isotopic content, is defined as the specific activity of the aflatoxin-B_1/specific activity of the labeled precursor added, based on the L isomer.
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tion at 30°, the aflatoxins were extracted and separated chromatographically.[1] The RIC of the aflatoxin-B_1 was less than 0.01, whereas if phenylalanine were a precursor of aflatoxin an RIC of 1.0 would be expected. Thus, phenylalanine is clearly excluded as a precursor of aflatoxin B_1.

The possible involvement of shikimic acid was examined in a similar fashion using the parent strain and measuring the incorporation of labeled shikimic acid into aflatoxin B_1 as compared to its incorporation into the phenylalanine in the cellular protein. Since shikimic acid is an intermediate of aromatic amino acid biosynthesis,[5] if it is also an intermediate in aflatoxin biosynthesis comparable specific activities should be observed when the cells are grown in the presence of [ring-^{14}C]shikimic acid. Following growth, the aflatoxins were extracted and separated, and the cells were dried and hydrolyzed with HCl. The hydrolysate was dried and dissolved in 10% aqueous 2-propanol. Aliquots were chromatographed on silica gel G plates, and the phenylalanine was measured by a comparison with standards after spraying with ninhydrin. The radioactivity of the phenylalanine was measured by liquid scintillation counting of the spot after scraping into vials. The specific activity of the phenylalanine was found to be 11 μCi/mmol, while that of the aflatoxin-B_1 was 0.21 μCi/mmol. This indicates that shikimic acid is not a precursor of aflatoxin B_1.

As [1-^{14}C]acetate has been found[1] to label aflatoxin-B_1 efficiently, it appeared likely that the molecule was derived in large part from acetate units. To examine this possibility, aflatoxin-B_1 was prepared from [1-^{14}C]- and [2-^{14}C]acetate by a procedure modified from that of Adye and Mateles[1] as follows.

Conidia of the parent strain were cultivated in 100 ml of synthetic medium[1] in 500-ml baffled erlenmeyer flasks at 25° on a rotary shaker at 200 rpm for 3 days. Mycelial pellets were collected on cheese cloth in a Büchner funnel, washed with distilled water, dispersed in distilled water with a Waring blender, refiltered, and resuspended in nitrogen-free resting cell medium. The suspension was filtered again, and 1 g of wet cake (containing about 12% dry cell mass) was resuspended in 10 ml of resting cell medium containing 50 μmol of glucose and 20 μmol of radioactive acetate. The culture was incubated at 25° for 12 hr on a rotary shaker at 250 rpm. The culture was filtered and the filtrate was extracted with chloroform. The washed chloroform extract was evaporated with a stream of nitrogen, and the aflatoxins were separated by thin layer chromatography.[1] In a typical preparative run, 1 mCi of [1-^{14}C]-acetate yielded 57 μCi of aflatoxin-B_1 with an RIC of 1.48 (hypothetical volume 9). Such highly active aflatoxin was subjected to chemical degradation[6] or used for metabolic studies in animals.[7,8]

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Intramolecular Exchange in Phosphorus Pentahalide Molecules[1]

Sir:

Pseudorotation of a trigonal-bipyramidal intermediate has been postulated recently by a number of investigators[2-5] concerned with various aspects of phosphorus chemistry. We wish to report results defining the requirements for such a process for phosphorus pentahalide molecules (known to undergo intramolecular exchange) and provide useful criteria in analyzing possible related situations. What is being considered here is a process analogous in some respects to the inversion occurring in the ammonia molecule.[6] In the case of trigonal-bipyramidal molecules an internal vibration is thought to lead to exchange of equatorial and axial positions, leaving the molecule rotated compared to its original state (Figure 1). This type of process was first postulated by Berry[7]

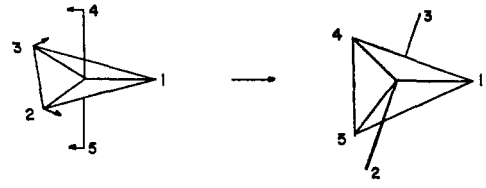
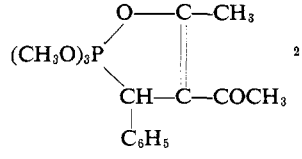


Figure 1. Pseudorotation.

to rationalize the appearance of equivalent fluorine environments in the ^{19}F nmr spectrum[8] of trigonal-bipyramidal PF_5. Nmr studies have now revealed examples of several trigonal-bipyramidal phosphorus compounds, PCl_2F_3,[9,10] PH_2F_3,[11] $(C_2H_5)_2NPF_4$,[10b] and



which have been shown to undergo exchange. In each case, a low-temperature pattern consistent with a nonexchanging structure transforms on increasing the temperature to a pattern showing averaging of fluorine atom magnetic environments with retention of P–F spin coupling.

Detailed considerations[12] of a vibrational exchange (pseudorotation), using a potential function[13] based on

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