Table III

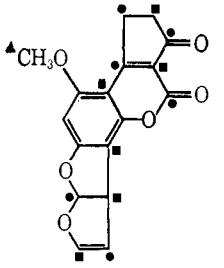
Carbon atoms	Estimated as	% of total radioactivity
C-1–C-7	*p*-Bromophenacyl *cis*-2-methylcyclopentanoate	43.84
C-7	CO_2	0.14
C-1, C-2	*p*-Bromophenacyl acetate	10.37; 11.05
C-2	CO_2	0.21
C-1	CO_2	8.52
C-1–C-6	*p*-Bromophenacyl caproate	44.48
C-6	CO_2	8.89
C-1–C-5	*p*-Bromophenacyl valerate	32.57
C-5	CO_2	6.77
C-1–C-4	*p*-Bromophenacyl butyrate	18.57
C-1–C-3	*p*-Bromophenacyl propionate	21.80
C-17	$CH_3N^+(C_2H_5)_3I^-$	0.32

Experimental support for the presence of seven labels (theoretical activity 14.3% per labeled carbon atom) in radioactive aflatoxin-B_1 prepared from acetate-2-^{14}C was secured again by degradations, outlined in Schemes II and III, and the results are summarized in Table IV.

Table IV

Carbon atoms	Estimated as	% of total radio-activity
C-11, C-13–C-16	*p*-Bromophenacyl 2-methylbutanoate	43.26
C-11	CO_2	12.83
C-13	CHI_3	0.49
C-14–C-16	*p*-Bromophenacyl propionate	28.61
C-14	CO_2	12.78
C-15	CO_2	0.49
C-16	CO_2	12.72
C-17	$CH_3N^+(C_2H_5)_3I^-$	0.31
C-1–C-7	*p*-Bromophenacyl *cis*-2-methylcyclopentanoate	43.06
C-7	CO_2	12.86
C-1, C-2	*p*-Bromophenacyl acetate	14.10
C-1	CO_2	0.35
C-2	CO_2	12.62

The resulting distribution of labels portrayed in formula **9** is not in accord with that predicted by[5–7] or implied[8] in a number of purely speculative schemes.



9

CH_3COOH

$CH_3SCH_2CH_2CH(NH_2)COOH$

(5) D. P. Moody, *Nature*, **202**, 188 (1964).
(6) J. G. Heathcote, J. J. Child, and M. F. Dutton, *Biochem. J.*, **95**, 23P (1965).
(7) R. Thomas in "Biogenesis of Antibiotic Substances," Z. Vaněk and Z. Hoštálek, Ed., Academic Press, New York, N. Y., 1965, p 155.
(8) J. S. E. Holker and J. G. Underwood, *Chem. Ind.* (London), 1865 (1964).

A new hypothesis consistent with this labeling pattern is presented in the accompanying communication.[9]

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(9) M. Biollaz, G. Büchi, and G. Milne, *J. Am. Chem. Soc.*, **90**, 5019 (1968).

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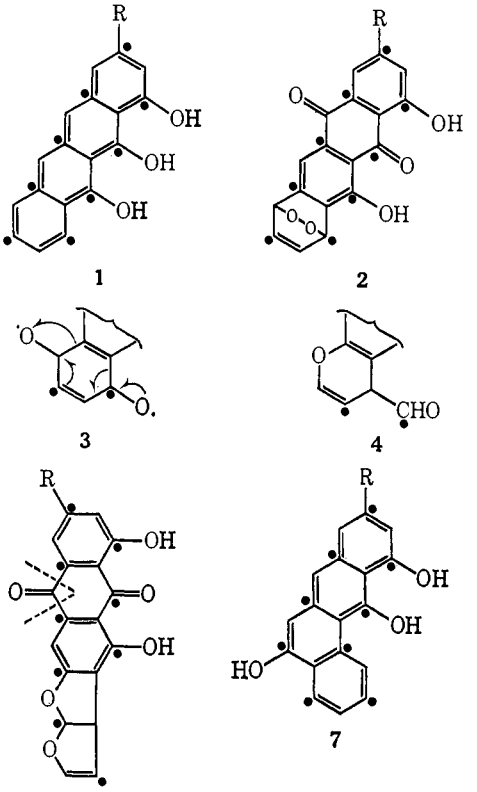
The Biogenesis of Bisfuranoids in the Genus *Aspergillus*

Sir:

In an accompanying communication[1] we reported degradative studies on radioactive aflatoxin-B_1 prepared by feeding experiments with labeled acetate (1-^{14}C and 2-^{14}C) and with methionine. The origin of 13 of the 17 carbon atoms present in aflatoxin-B_1 was determined, and the distribution of labels is indicated in formula **10**. We wish to propose a hypothetical scheme for the biogenesis of the aflatoxins and related mold metabolites which is consonant with the experimental evidence now in hand. It is assumed that the acetate-derived polyhydroxynapthacene **1** (R = H or OH) is oxidized to the *endo*-peroxyanthraquinone **2** which rearranges *via* the



1 **2** **3** **4** **7**

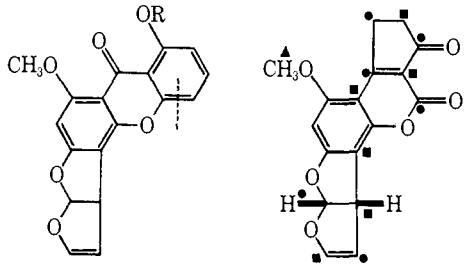
5, R = OH
6, R = OCH_3

(1) M. Biollaz, G. Büchi, and G. Milne, *J. Am. Chem. Soc.*, **90**, 5018 (1968).

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.

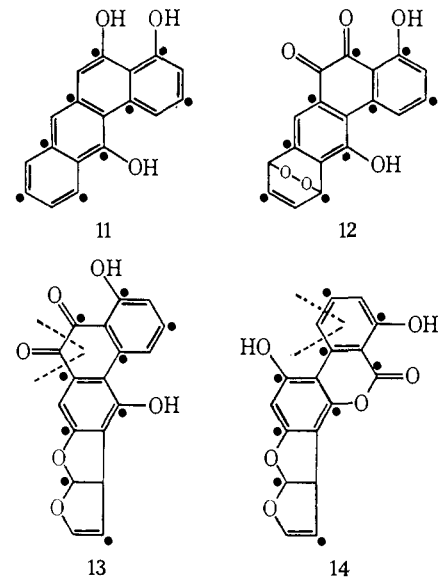


8, R = H
9, R = CH_3

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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