also prepared. From the small shifts of the cytidine amino protons we conclude that the equilibrium constant for complex formation is at least an order of magnitude less for methylguanosine–cytidine dimer formation than the value for guanosine–cytidine dimer formation. Substitution of a methyl group at the N_1 position eliminates one of the three hydrogen bonds in the GC dimer (see Figure 2), but steric hindrance from the methyl should prevent more than one hydrogen bond from forming. Thus it is not surprising that little or no hydrogen bonding is observed. Pochon and Michelson draw similar conclusions from their results on the interaction of poly 1-methyl-G with poly C.[32]

Optical Studies. An attempt was made to directly observe the formation of GC base pairs in DMSO by the use of circular dichroism (CD). It is known that the poly G–poly C double strand complex has an optical rotatory dispersion curve quite different from the sum of the rotatory dispersion of the two polymer strands measured separately.[33,34] There must be a corresponding large change in the circular dichroism. The CD of a solution which contains 0.045 *M* guanosine and cytidine is slightly different from the CD measured for guanosine and cytidine separately in DMSO. A very weak new positive CD band appears upon interaction which is centered at 285 mμ. At this concentration the equilibrium constant derived from the nmr results implies that 13% of the guanosine and cytidine should be hydrogen bonded. Solutions of 5′-trityl-2′,3′-benzylidylguanosine and 5′-trityl-2′,3′-benzylidylcytidine show a large change in CD when the substituted nucleosides are allowed to interact in $CHCl_3$. Here the equilibrium constant for interaction is considerably larger. A new positive CD band appears which is centered at 290 mμ. Some of the CD change may result from interaction between the aromatic derivatives and the bases and further optical studies in these systems are in progress. However, the similarity of the CD bands due to interaction in DMSO and $CHCl_3$ is reasonable evidence that we are observing similar hydrogen-bonded complexes in both cases.

(32) F. Pochon and A. M. Michelson, *Biochem. Biophys. Acta*, **145**, 321 (1967).
(33) P. L. Sarkar and J. T. Yang, *Biochemistry*, **4**, 1238 (1965).
(34) C. R. Cantor, S. R. Jaskunas, and I. Tinoco, Jr., *J. Mol. Biol.*, **20**, 39 (1966).

Conclusions

The high dielectric constant (45) and strong proton acceptor properties of DMSO make it much more similar to water than $CHCl_3$. The extent of hydrogen bonding between bases is much less in DMSO than in $CHCl_3$. Pitha, *et al.*, have determined an equilibrium constant of over 10^5 for hydrogen bonding between guanosine and cytosine derivatives in $CHCl_3$ from infrared measurements.[13] We find an equilibrium constant of about 4 for the association of guanosine and cytidine in DMSO. In water the degree of association is so weak that direct observation of GC complexes has not yet been achieved.

Kyogoku, *et al.*, have observed an enthalpy change of -6.2 ± 0.6 kcal/mol associated with the formation of a complex between 9-ethyladenine and 1-cyclohexyluracil in $CHCl_3$. If this enthalpy is attributed entirely to hydrogen bonding the result is -3.1 kcal/mol per hydrogen bond. Our results of -5.8 kcal/mol for GC pairing in DMSO in turn yield -1.9 kcal/mol per hydrogen bond. The moderately large negative enthalpy found in DMSO may indicate that the enthalpy of hydrogen bond formation between guanosine and cytidine is still negative in aqueous solution. The entropy change found for dimer formation between guanosine and cytidine in DMSO was -16 eu. This compares reasonably well with the value of -11 eu found by Kyogoku, *et al.*, for AU pairing in $CHCl_3$.

The structure of the GC pair in DMSO is almost certainly the same as the base-pairing scheme known to occur in double strand DNA. Formation of G_2 and C_2 pairs was also observed in DMSO. These complexes are much weaker than GC. Some information about the structure of these complexes can be obtained from the nmr results, but it is not yet possible to select a unique base-pairing scheme for them.

Acknowledgment. The Varian HA-100 nmr spectrometer was provided by an institutional grant from the National Science Foundation to the University of Colorado. This work was supported by Grant GM-14825 from the U. S. Public Health Service. We are grateful to Dr. Joan F. Newmark for assistance in preparing the solutions, and to Miss Maria Wierzbicka for carrying out some preliminary nmr studies.

Communications to the Editor

Biosynthesis of Aflatoxins

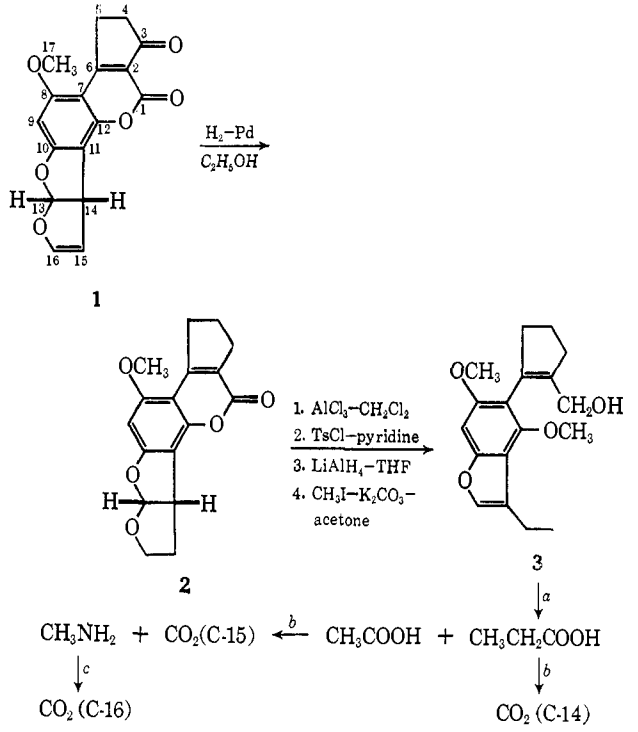
Sir:

The aflatoxins are a structurally and biologically remarkable group of metabolites produced by some *Aspergillus* species.[1,2] Since their origin in nature is not obvious from inspection of their structures, we examined the biosynthesis of aflatoxin-B_1 with the aid of radioactive precursors. Administration[2] of methionine-methyl-^{14}C to *Aspergillus flavus* yielded radioactive aflatoxin-B_1 (1). Zeisel degradation gave methyl iodide containing 97.8% of the activity of the starting material.[3]

Aflatoxin-B_1 prepared from acetate-1-^{14}C[4] was de-

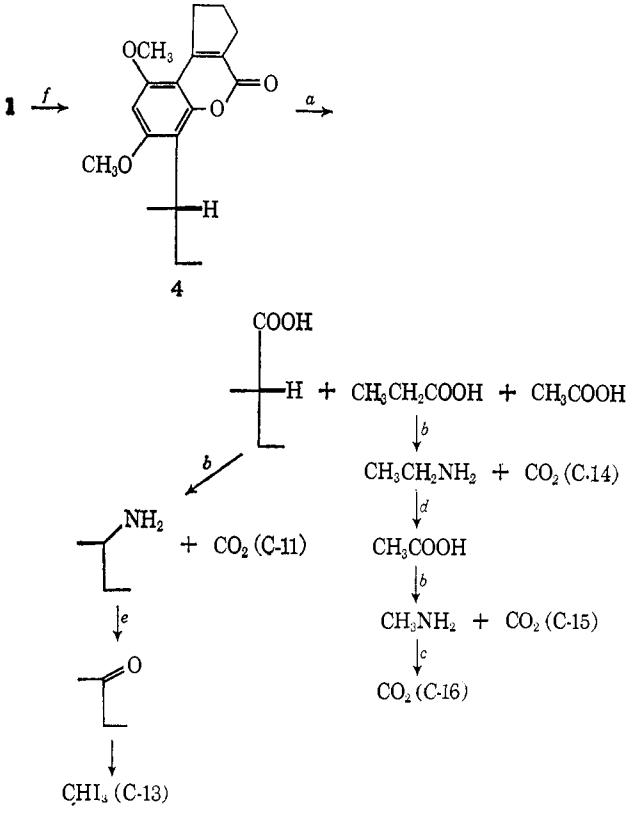
(1) G. N. Wogan, *Bacteriol. Rev.*, **30**, 460 (1966).
(2) R. I. Mateles and G. N. Wogan, *Advan. Microbial. Phys.*, **1**, 25 (1967).
(3) We are indebted to Dr. S. Brechbühler for this determination.
(4) J. A. Donkersloot, D. P. H. Hsieh, and R. I. Mateles, *J. Am. Chem. Soc.*, **90**, 5020 (1968).

Scheme I



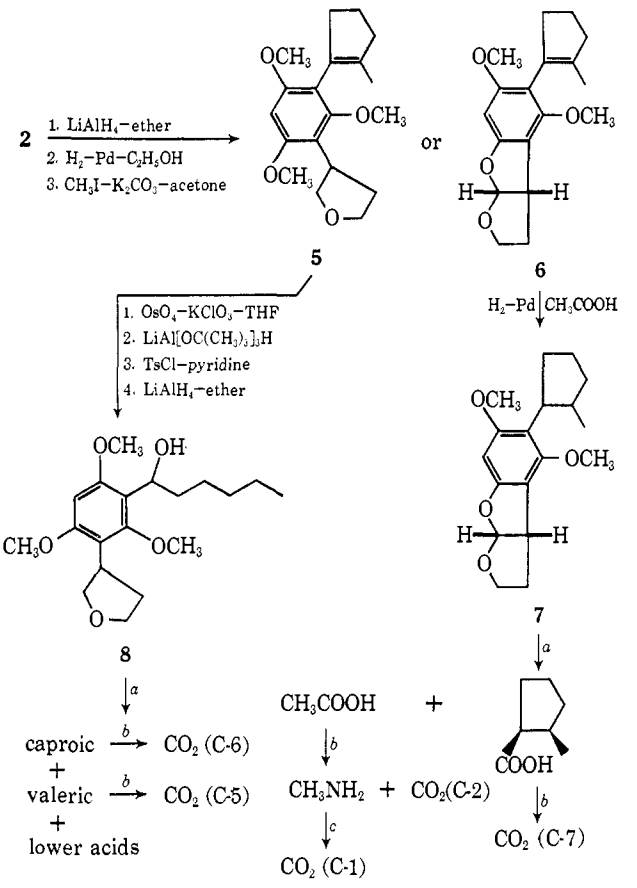
[a] Kuhn-Roth oxidation: E. Wiesenberger, *Mikrochim. Acta*, **33**, 51 (1948). [b] Schmidt degradation: J. J. Britt, Dissertation, Eidgenössischen Technisches Hochschule, Zurich, 1959. [c] Oxidation with $KMnO_4$: J. J. Britt, Dissertation.

Scheme II



[a–c] As in Scheme I. [d] $KMnO_4$ oxidation: S. Brechbühler, Dissertation, Eidgenössischen Technisches Hochschule, Zurich, 1964. [e] NaOCl oxidation: H. Ruschig, W. Fritsch, J. Schmidt-Thomé, and W. Haede, *Ber.*, **88**, 883 (1955). [f] S. Brechbühler, G. Büchi, and G. Milne, *J. Org. Chem.*, **32**, 2641 (1967).

Scheme III



[a–c] As in Scheme I.

graded using the three separate sequences outlined in Schemes I–III, and the distribution of radioactivity found is summarized in Tables I–III.

Table I

Carbon atoms	Estimated as	% of total radioactivity
C-14–C-16	*p*-Bromophenacyl propionate	12.01
C-14	CO_2	1.35
C-15, C-16	*p*-Bromophenacyl acetate	11.21
C-15	CO_2	8.61
C-16	CO_2	1.21

Table II

Carbon atoms	Estimated as	% of total radioactivity
C-11, C-13–C-16	N-α-Naphthyl-2-methylbutanamide	22.31
C-11	CO_2	0.13

These results establish specific incorporation, and comparison of measured and calculated radioactivities demands the presence of nine labels (theoretical activity 11.1% per labeled carbon atom) in acetate-1-^{14}C-derived aflatoxin-B_1. If it is assumed that the metabolite originates from a nonaacetyl chain, two methyl-derived carbon atoms must have been eliminated.

Table III

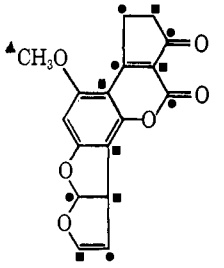
Carbon atoms	Estimated as	% of total radioactivity
C-1–C-7	*p*-Bromophenacyl *cis*-2-methylcyclopentanoate	43.84
C-7	CO_2	0.14
C-1, C-2	*p*-Bromophenacyl acetate	10.37; 11.05
C-2	CO_2	0.21
C-1	CO_2	8.52
C-1–C-6	*p*-Bromophenacyl caproate	44.48
C-6	CO_2	8.89
C-1–C-5	*p*-Bromophenacyl valerate	32.57
C-5	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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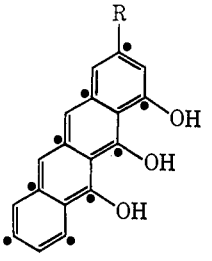
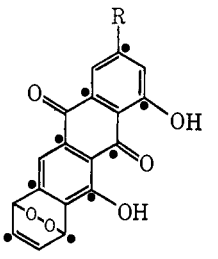
The Biogenesis of Bisfuranoids in the Genus *Aspergillus*

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



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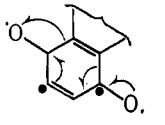

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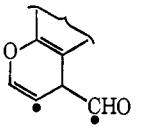

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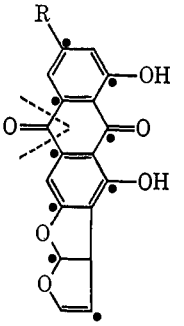
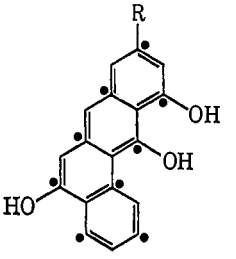


5, R = OH
6, R = OCH_3



7

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