mechanism of metal oxidation and alkyl migration is currently under investigation.

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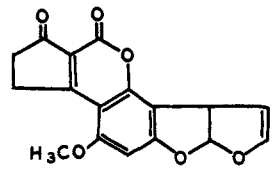
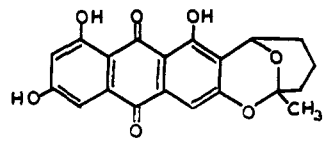
Averufin in the Biosynthesis of Aflatoxin B_1

Sir:

Chemical degradation[1] of aflatoxin B_1 (I) prepared from ^{14}C-labeled acetate by cultures of *Aspergillus parasiticus* ATCC 15517[2] has revealed that the basic skeleton of the aflatoxin molecule is totally derived from acetate units and that methionine contributes the methoxy methyl group. Based on the label distribution in the molecule, a number of fungal metabolites have been proposed as precursors of aflatoxin B_1.[3]



I



II

Recently, a mutant of *A. parasiticus* ATCC 15517 impaired in aflatoxin biosynthesis has been found to accumulate averufin (II),[4] a fungal pigment not being included in the above proposed intermediates for aflatoxin biosynthesis. ^{14}C-Labeled averufin was thus prepared from cultures of the mutant supplemented with [1-^{14}C]acetate, and was used to test if averufin cannot be incorporated into aflatoxin B_1 by the wild type cells.

Conidia of the deficient mutant, W49,[4] were cultivated in a synthetic medium[5] supplemented with 0.1% yeast extract as shaken cultures. Mycelial pellets at 48 hr were harvested to prepare resting cell cultures[6] of the mutant, which was then fed [1-^{14}C]acetate and incubated to accumulate [^{14}C]averufin. The labeled averufin was exhaustively extracted from the mycelial pellets with acetone and purified successively using three tlc systems: ChromAR 500 sheets (Mallinckrodt Chemical Works, St. Louis, Mo.) developed with chloroform–acetone–*n*-hexane (85:15:20, CAH), benzene–ethanol–water (46:35:19, BEW), and benzene–petroleum ether–acetone (90:8:2, BPeA). The purity of [^{14}C]averufin was verified by coincidence of spots in chromatograms and autoradiograms and by comparing the uv spectrum of the [^{14}C]averufin with that of pure averufin obtained from Donkersloot.[4]

The resting cell techniques used to incorporate [1-^{14}C]acetate into aflatoxin[7] were employed in the incorporation studies. [^{14}C]Averufin (1.5 μmol, 0.3 μCi/μmol) dissolved in 0.2 ml of acetone was placed in each 50-ml baffled flask to which was then added slowly 9.8 ml of the resting cell medium.[7] By this procedure 68% of the [^{14}C]averufin remained dissolved in the aqueous medium. One gram of wet cells was added to each flask and the flasks shaken (150 rpm) for 20 hr at 30°. Control flasks containing 30 μmol of [1-^{14}C]-acetate and 2% acetone were used to check the *de novo* synthetic activity of the cells. Acetone at this concentration (2%) reduced the incorporation efficiency of [1-^{14}C]acetate into aflatoxins by 37%. Another set of control flasks containing autoclaved cells was used to demonstrate the enzyme activities involved in the label incorporation.

In the first experiment, 150 μg of unlabeled aflatoxin B_1 was added to each flask as a carrier to facilitate extraction and purification. Aflatoxins were extracted from the fermented broths with chloroform and were separated from residual averufin and other compounds successively using two tlc systems: ChromAR-500 sheets developed with ethyl acetate–isopropyl alcohol–water (10:2:1, EaIpW) and BPeA. Aflatoxins eluted with chloroform from the ChromAR strips were further purified twice on Adsorbosil-1 plates developed with CAH and EaIpW. The purified aflatoxin B_1 retained 8.4 and 0.6% of the labels from [^{14}C]averufin and [1-^{14}C]-acetate, respectively. To ascertain that the radioactivity in aflatoxin B_1 was not due to the contamination of radioactive impurities, aflatoxin B_1 was subjected to further purification with two different tlc systems. The specific radioactivity of aflatoxin B_1 remained constant after each purification. The aflatoxin B_1 similarly isolated and purified from the control flasks containing deactivated cells did not contain any radioactivity.

In the second experiment, no carrier aflatoxin B_1 was added to the flasks and the labeled aflatoxin B_1 was isolated and purified by the same series of tlc systems. The relative specific activity or RIC[8] of the aflatoxin B_1 derived from [^{14}C]averufin was more than twice that of aflatoxin B_1 from [1-^{14}C]acetate, despite the fact that averufin concentration in the medium was much lower than acetate.

The above results as well as the accumulation of averufin by the deficient mutant suggested that averufin is a precursor of aflatoxin B_1. To confirm this, coexistence of averufin and aflatoxin in the parent strain culture was examined. Fermented broths and mycelia of *A. parasiticus* ATCC 15517 which had been grown for 48 hr in synthetic medium[5] were extracted with

(1) M. Biollaz, G. Büchi, and G. Milne, *J. Amer. Chem. Soc.*, **90**, 5017 (1968).
(2) J. A. Donkersloot, D. P. H. Hsieh, and R. I. Mateles, *ibid.*, **90**, 5020 (1968).
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(4) J. A. Donkersloot, R. I. Mateles, and S. S. Yang, *Biochem. Biophys. Res. Commun.*, **47**, 1051 (1972).
(5) J. C. Adye and R. I. Mateles, *Biochem. Biophys. Acta*, **86**, 418 (1964).
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(8) RIC, relative isotope content, shows the precursor–product relationship of a labeled compound with aflatoxin. The higher the RIC value the closer is the product to the precursor. See ref 7 for details.

chloroform and acetone, respectively, and the aflatoxins and averufin were separated and purified by the tlc systems described above. The parent strain culture was found to indeed produce averufin though in small quantity (1 μmol/l. as compared to 43 μmol/l. of aflatoxin B_1). To ascertain the coexistence of the two metabolites, a resting cell culture of the parent wild type strain was incubated with [1-^{14}C]acetate for 10 hr, and 60 μg of unlabeled pure averufin was added to each flask as carrier. After extraction and purification, 1.32 and 0.41% of the labels in [1-^{14}C]acetate were found in the recovered aflatoxin B_1 and averufin, respectively. The relative specific radioactivity, or RIC, of aflatoxin B_1 so obtained was only one-tenth that of the averufin, indicating that averufin is subject to less extent of isotope dilution and hence is an intermediate in the pathway leading from acetate to aflatoxin B_1.

Averufin has been proposed by Thomas[9] as an intermediate in a hypothetical pathway for the biosynthesis of aflatoxin from acetate, involving sterigmatocystin. This pathway, however, does not account for the distribution of labels in the aflatoxin B_1 molecule biosynthesized from [^{14}C]acetate.[1] The biosynthetic scheme proposed by Biollaz, *et al.*,[3] based upon the labeling pattern in turn does not imply involvement of averufin. Therefore, a pathway different from either of the previously proposed pathways must actually be used in the biosynthesis of aflatoxin B_1.

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(9) R. Thomas, "Biogenesis of Antibiotic Substances," Z. Vanek and Z. Hestalek, Ed., Academic Press, New York, N. Y., 1965, p 155.

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Lithium Triethylborohydride. An Exceptionally Powerful Nucleophile in Displacement Reactions with Organic Halides

Sir:

Lithium triethylborohydride possesses enormous nucleophilic power in SN2 displacement reactions with alkyl and cycloalkyl halides (eq 1). Kinetic studies

$$LiEt_3BH + RX \longrightarrow RH + Et_3B + LiX \quad (1)$$

reveal that the reagent is considerably more powerful than nucleophiles such as thiophenoxide and alkyl mercaptide, previously considered to be the most powerful simple nucleophiles available for SN2 displacements[1] (eq 2).

$$k_{Et_3BH^-}/k_{C_6H_5S^-} = 20$$
$$k_{Et_3BH^-}/k_{NO_3^-} = 10^7 \quad (2)$$

(1) For a detailed review and discussions on the subject of nucleophilic reactivity see: (a) J. F. Bunnett, *Annu. Rev. Phys. Chem.*, **14**, 271 (1963); (b) A. Streitwieser, Jr., "Solvolytic Displacement Reactions," McGraw-Hill, New York, N. Y., 1962, pp 9-11; (c) J. O. Edwards and R. G. Pearson, *J. Amer. Chem. Soc.*, **84**, 16 (1962); (d) R. G. Pearson, H. Sobel, and J. Songstad, *ibid.*, **90**, 319 (1968).

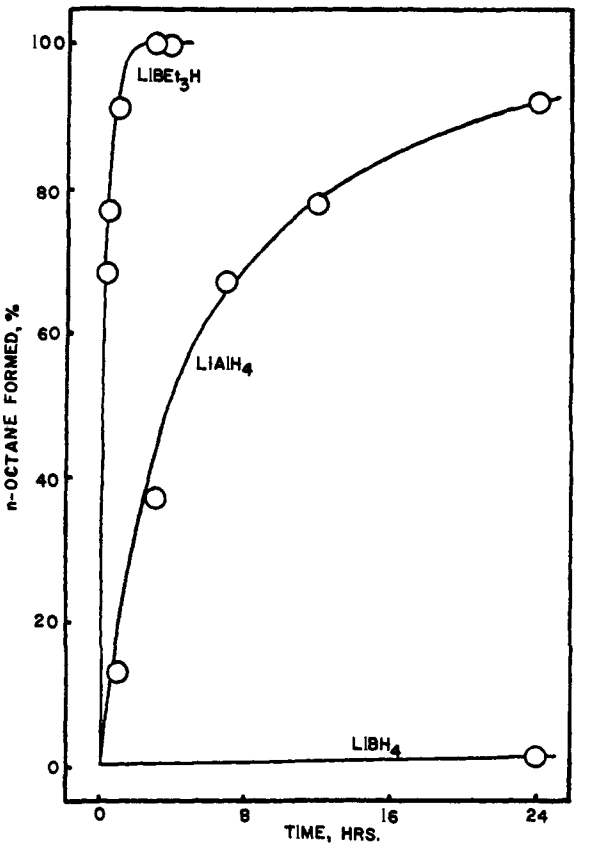

Figure 1. Rates of reduction of *n*-octyl chloride (0.25 *M*) with representative complex metal hydrides (0.5 *M*) in tetrahydrofuran at 25°.

Addition of triethylborane to THF solutions of lithium tri-*tert*-butoxyaluminohydride (LTBA) results in a rapid disappearance of hydride and the concurrent formation of 1-butanol.[2] It was established that this reductive cleavage proceeds through the formation of lithium triethylborohydride[3] and monomeric aluminum *tert*-butoxide.[4]

In the course of these investigations, certain observations suggested that lithium triethylborohydride must be an exceptionally powerful nucleophile. To test this possibility, lithium triethylborohydride was prepared in THF (eq 3) and treated with *n*-octyl chloride. Com-

$$LiH + BEt_3 \xrightarrow[25°]{THF} LiEt_3BH \quad (3)$$

parison of the rate of reduction with lithium aluminum hydride and lithium borohydride revealed the marked superiority of the new reagent (Figure 1).

These results persuaded us to undertake a quantitative comparison of the rates of reaction of lithium triethylborohydride and sodium thiophenoxide with *n*-octyl chloride under identical conditions. The results revealed that the reaction involving lithium triethylborohydride is far faster (Table I).

From these results, we calculated the nucleophilic reactivity of other nucleophiles using Streitwieser's nucleophilicity data:[1] Et_3BH^-, 9,400,000; n-$C_4H_9S^-$,

(2) H. C. Brown, S. Krishnamurthy, and R. A. Coleman, *ibid.*, **94**, 1750 (1972).
(3) H. C. Brown, H. I. Schlesinger, I. Sheft, and D. M. Ritter, *ibid.*, **75**, 192 (1953).
(4) H. C. Brown and S. Krishnamurthy, *J. Chem. Soc., Chem. Commun.*, 868 (1972).