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# Anthraquinones in the Biosynthesis of Sterigmatocystin by *Aspergillus versicolor*

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$^{14}$C-labeled averufin, versiconal hemiacetal acetate, and versicolorin A were efficiently converted to sterigmatocystin by *Aspergillus versicolor*, thus providing experimental evidence that these anthraquinones are biosynthetic precursors of sterigmatocystin, a xanthone.

Radiotracer studies using $^{14}$C-labeled averufin (5), an orange pigment recently identified as versiconal hemiacetal acetate (1, 9) and versicolorin A (4) have shown that these polyhydroxyanthraquinones are convertible to aflatoxins by cultures of *Aspergillus parasiticus*, and that they are biosynthetic intermediates in aflatoxin biosynthesis. Sterigmatocystin, a xanthone, was also readily convertible into aflatoxins (2). The structures of averufin (I), versiconal hemiacetal acetate (II), versicolorin A (III), and sterigmatocystin (IV), are shown in Fig. 1. Our recent work (8) has shown that all three of these anthraquinones precede IV in the pathway of aflatoxin biosynthesis in *A. parasiticus*. This communication presents evidence that IV can be derived from III, II, and I by cultures of *A. versicolor*.

$^{14}$C-labeled averufin and versiconal hemiacetal acetate were synthesized from [1-$^{14}$C]acetate by using resting cell cultures of producing strains as described previously (9). Versicolorin A was produced by using a mutant strain of *A. parasiticus* as outlined by Lee et al. (4). To study the conversion of various precursors, conidia of *A. versicolor* ATCC 18643 (*A. versicolor* NRRL 5219 in the case of versicolorin A) were inoculated into 10 ml of asparagine-low salt medium (7) contained in 50-ml flasks and incubated without shaking at 28°C. After 5 days of incubation (11 days in the case of strain NRRL 5219), when the mycelial mat had turned dark-brown in color and IV production had begun, 0.2 ml of a methanolic solution of appropriate precursor was added to the flask. The organisms with radiolabeled precursors were allowed to incubate for 3 to 5 additional days. IV in the mycelia and medium was then extracted with acetone and chloroform, respectively. The two extracts were pooled and dried in vacuo. The residue was suspended in chloroform, and IV was purified to a constant specific activity by repeated thin-layer chromatography using Silica Gel G plates, and chloroform-acetone-water (88:12:15, vol/vol/vol), hexane-acetone-ethyl ether (70:30:20, vol/vol/vol), and benzene-ethylacetate-isopropanol-water (25:10:2:1, vol/vol/vol/vol) as developing solvents, respectively.

The incorporation of radioactivity from various $^{14}$C-labeled precursors into IV is presented in Table 1. The efficient incorporation of [1-$^{14}$C]acetate into IV indicates that the organism had a normal de novo synthetic activity. The radioactivity from all three anthraquinones was even more efficiently incorporated into IV. After 3 to 5 days of incubation, averages of 16.52, 17.50, and 38% of radioactivity was retained in IV from averufin, versiconal hemiacetal acetate, and versicolorin A, respectively. Due to low solubility of the precursors in the aqueous medium, the anthraquinones tended to precipitate out of solution in standing culture, thereby making their uptake by the cells especially difficult. In calculating incorporation efficiencies, no corrections for the net uptake of the precursors were made. Thus even a higher percent incorporation could be observed if the amounts of the unutilized precursor were accounted for.

The relative specific activities (RSA) of IV derived from acetate, averufin, versiconal hemiacetal acetate, and versicolorin A were 0.011, 0.074, 0.14, and 0.43, respectively. The RSA, which is dependent on precursor concentration, is an indication of precursor-product relationship (3)—the higher the RSA value, the closer is the product to the precursor in the pathway. Thus, an increasing trend in RSA values of IV derived from various precursors confirms their sequence in the biosynthetic pathway of IV, i.e., acetate → averufin → versiconal hemiacetal ace-

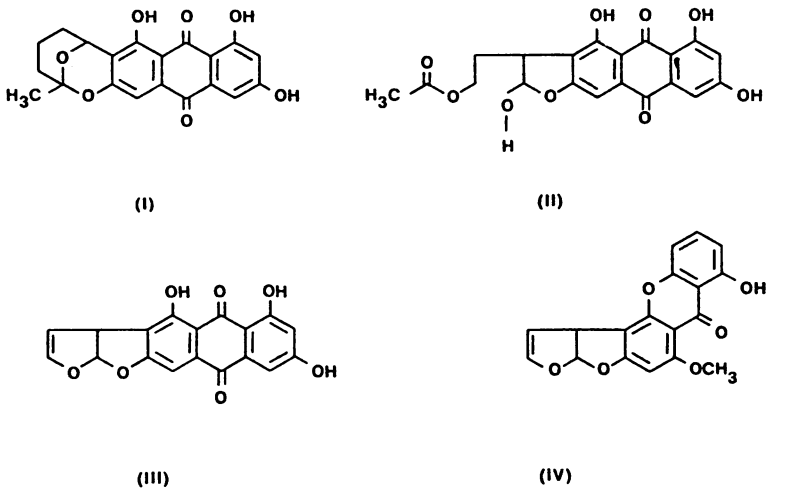


FIG. 1. *Structures of averufin (I), versiconal hemiacetal acetate (II), versicolorin A (III), and sterigmatocystin (IV).*

TABLE 1. *Incorporation of $^{14}C$-labeled acetate, averufin, versiconal hemiacetal acetate, and versicolorin A into sterigmatocystin by Aspergillus versicolor*[a]

| $^{14}C$ precursor[b] | Amt | | Incorporation efficiency (%) | Sterigmatocystin | |
|---|---|---|---|---|---|
| | μmol | dpm × $10^4$ | | Sp act (Ci/mol) | RSA[c] |
| Acetate | 6.42 | 9.49 | 0.85 | 0.007 | 0.011 |
| Averufin | 6.55 | 8.59 | 16.52 | 0.006 | 0.074 |
| Versiconal hemiacetal acetate | 6.54 | 13.16 | 17.50 | 0.009 | 0.14 |
| Versicolorin A | 1.78 | 9.95 | 25.38 | 0.025 | 0.43 |

[a] Results are the averages of triplicate experiments.

[b] Concentrations and specific activities of the precursors used were: acetate, 8 mM (0.632 Ci/mol); averufin, 0.292 mM (0.81 Ci/mol); versiconal hemiacetal acetate, 0.532 mM (0.065 Ci/mol); versicolorin A, 0.304 mM (0.058 Ci/mol).

[c] RSA, Relative specific activity, i.e. specific radioactivity of product divided by specific radioactivity of precursor.

tate → versicolorin A → sterigmatocystin, as we have shown previously for aflatoxin biosynthesis (8).

It is of interest to note that the pathway of aflatoxin biosynthesis found in *A. parasiticus* also exists in *A. versicolor* but terminates at the formation of IV in the latter species. Although aflatoxin production is limited to *A. flavus* and *A. parasiticus,* sterigmatocystin is produced by a fairly large number of species (6). The distribution of this pathway among different fungal species, accumulating different metabolites in the pathway as end products, suggests that these compounds may have widespread occurrence in nature. If toxic, they could be mycotoxins of actual significance comparable to aflatoxins.

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