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Mycotoxins - Their Biosynthesis in Fungi: Biosynthesis of the Trichothecenes

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ABSTRACT

The biosynthetic pathway as currently postulated for trichothecin, a model 12,13-epoxy-Δ^9-trichothecene, involves the following sequence: 3 mevalonic acids → farnesyl pyrophosphate → trichodiene → trichodiol → 12,13-epoxytrichothec-9-ene → trichodermol → 12,13-epoxy,4β,8α-dihydroxy-trichothec-9-ene → trichothecolone → trichothecin.

The trichothecenes are a group of closely related sesquiterpenoid mycotoxins with cytotoxic, phytotoxic, antifungal, and insecticidal activity. Currently, there are almost 40 of these compounds known that occur naturally. All possess a 12,13-epoxy-Δ^9-trichothecene nucleus named after the first member of the group to be isolated--trichothecin. Trichothecenes are produced by a number of genera, but of most practical importance are those synthesized by the various *Fusarium* spp. and *Stachybotrys atra*. The structure, stereochemistry, and numbering system of trichothecin, an ester of isocrotonic acid, are shown in Fig. 1.

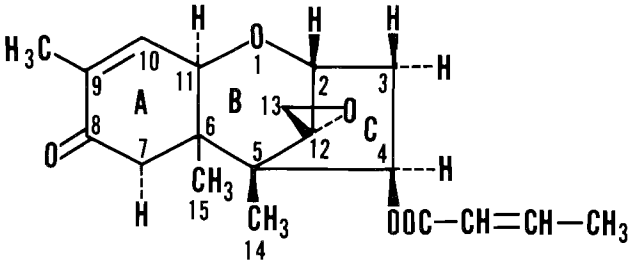

Figure 1. *Structure of trichothecin.*

It has been well established that the trichothecene skeleton is formed from three molecules of mevalonate via the usual pathway of lipid biosynthesis involving isopentenyl, geranyl, and farnesyl pyrophosphate (*2,3,6, 10,11,16,17,21-23,26*). The open chain farnesyl skeleton cyclizes to form the parent hydrocarbon of the trichothecene series, trichodiene (Fig. 2). In farnesyl pyrophosphate the configuration about the double bond at C6,7 may be cis or trans, which could result in two possible configurations on the initial folding of the molecule (Fig. 3). Achillidelis et al. (*1,2*) used two approaches to resolve this important problem. In feeding experiments with *Trichoderma sporulosum* and *Trichothecium roseum*, doubly labeled mevalonates of known ^3H:^{14}C ratio were employed, and the ratio of the two isotopes were determined in the metabolites and in their degradation products. From these data they could determine the number and location of the mevalonoid hydrogen atoms incorporated, thereby deducing information on the post-farnesyl pyrophosphate step. In the second approach, specifically labeled geranyl and farnesyl pyrophosphate were employed, thereby determining if a specific hydrogen or carbon atom originated in the distal, central, or terminal prenyl unit. This is illustrated in Fig. 3 where C-1,2 of farnesyl pyrophosphate were ^{14}C labeled. If the folding were cis, labels at C-1,2 would wind up at C-7,8 in the trichothecene nucleus; whereas if trans, labels would be found at C10,11. Achilladelis et al. showed experimentally that labeling occurred at C10,11, supporting the trans configuration of farnesyl pyrophosphate (Fig. 3).

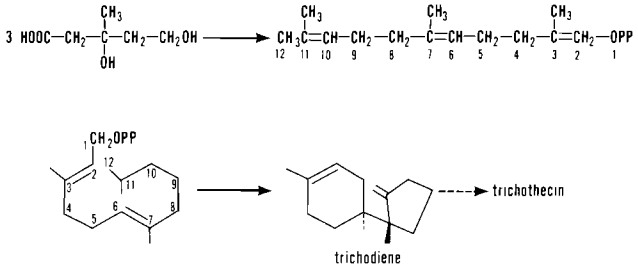

Figure 2. *General scheme for the biosynthesis of trichothecin.*

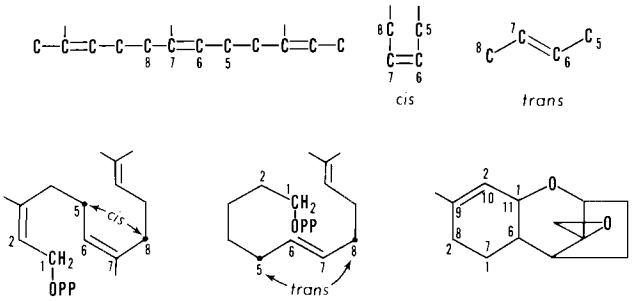

Figure 3. *The initial folding of farnesyl pyrophosphate.*

The structure of trichodiene (Fig. 4) was established by Nozoe and Machida (*24*) who postulated that it represented the biosynthetic precursor of the trichothecenes. Evans et al. (*13*) and Evans and Hanson (*12*) showed that to form the six-membered ring of

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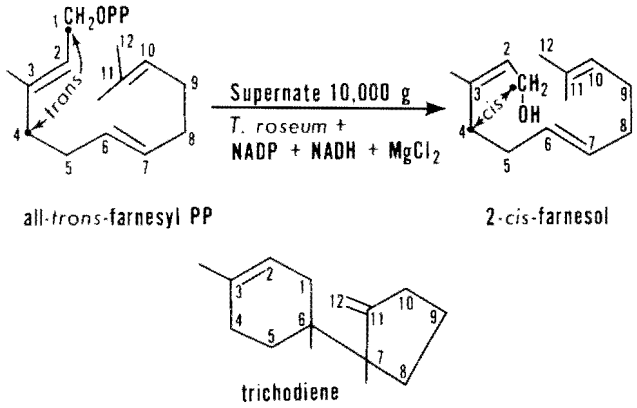

Figure 4. *Cyclization of farnesyl pyrophosphate to trichodiene.*

trichodiene, all-trans-farnesyl pyrophosphate must first be converted into a 2-cis-farnesyl unit. They used a cell-free system from 3-day-old shake cultures of *T. roseum* supplemented with reduced pyridine nucleotides to carry out the conversion (Fig. 4).

The sequence between farnesyl and trichothecin was confused until recently because what appeared to be an attractive intermediate, bis-aboline, had been proposed. However, labeling experiments of Achilladelis et al. (*2*) and the work of Nozoe and Machida (*24,25*), Machida and Nozoe (*18*), and Forrester and Money (*15*) eliminated this possibility. Instead, it was suggested that the cyclization of 2-cis-trans-farnesyl pyrophosphate may be concerted, with attack of an enzyme at C-10 initiating cyclization (Fig. 5). In the concerted cyclization sequence, a hydrogen transfer occurs from C_6 to C_{10} causing removal of the enzyme and also possibily initiating a methyl group rearrangement to trichodiene.

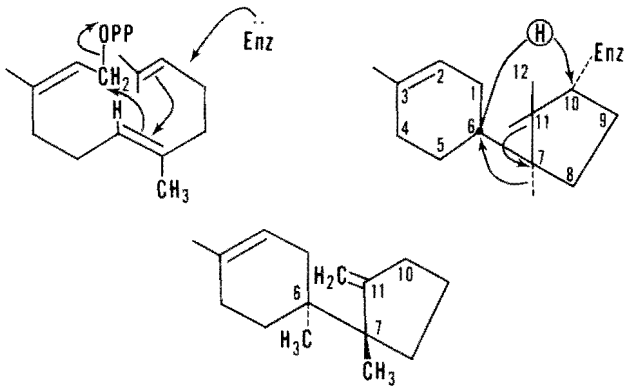

Figure 5. *Mechanism of cyclization of farnesyl pyrophosphate to trichodiene.*

Achilladelis and his colleagues (*2*) could explain the final structure of trichodiene by postulating two 1,2-methyl shifts. A double methyl group shift had previously been postulated to occur by Jones and Lowe (*17*) in their studies on the biosynthesis of trichothecin. In an earlier study, two 1,2-methyl shifts were demonstrated in the cyclization of squalene to lanosterol (*9,20*).

In 1970, Nozoe's group in Japan isolated from *T. roseum* a number of new trichothecene-type compounds that were of obvious biogenetic significance. Among these were trichodiene and trichodiol. The mechanism of formation of trichodiene to trichodiol has not been established, although it would be tempting to suggest hydroperoxide formation at C-11 as a fleeting intermediate (Fig. 6). The relationship between trichodiol and the more elaborate derivatives is clear since trichodiol needs only to cyclize at C-11 to give the trichothecene nucleus. Again, the mechanism of formation of the pyran nucleus has not been determined, and these experiments need to be done.

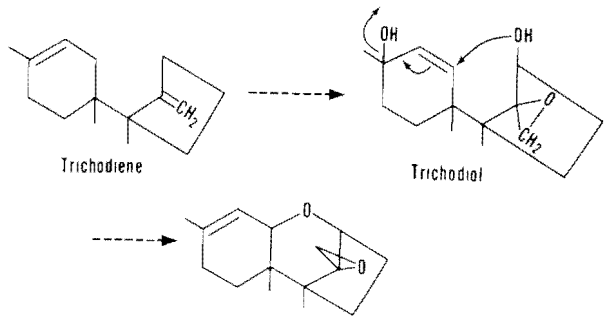

Figure 6. *Possible scheme for the biosynthesis of the trichothecene ring system.*

The carbonyl moiety at C-8 in trichothecin can be postulated to arise by a number of schemes (*14*). The most complex was that the C-8 and C-11 oxygen functions were related and that they arose by rearrangement of a Δ^9-8,11-epidioxide, itself formed from a ring A diene. There are precedents for this in a related series of compounds, the cuprenenes. A second hypothesis involved a 7,8 epoxide, such as found in crotocin, a trichothecene which co-occurs with trichothecin in *T. roseum*. A third proposal encompasses simple hydroxylation at C-8 followed by oxidation. These three proposals are summarized in Fig. 7.

Scheme 1 (Fig. 7) involving the epidioxide is ruled out because trichodermol, which lacks a C-8 carbonyl group but does have a -OH at C-4, is readily transformed to trichothecin by *T. roseum* (Fig. 8). This probably excludes oxygenation at C-8 before formation of the trichothecene skeleton and also shows that once the skeleton is formed, oxidation at C-4 precedes that at C-8.

In formation of a 7,8-epoxide as in crotocin (Fig. 7), hydrogen atoms would be removed from C-7 and C-8.

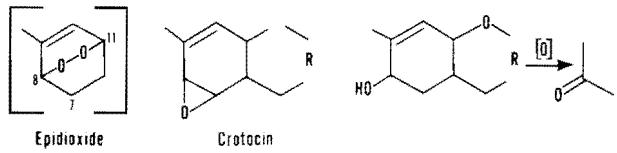

Figure 7. *Formation of a keto-moiety at carbon-8 in the trichothecene ring system.*

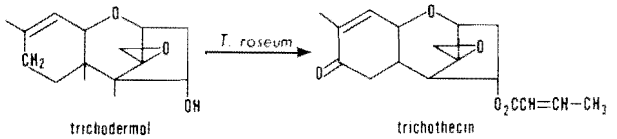

Figure 8. *Bioconversion of trichodermol to trichothecin.*

During the subsequent isomerization of the epoxide to form the carbonyl group, a C-8 hydrogen atom originating from C-2 of mevalonate would migrate to C-7 (Fig. 9). However, labeling data do not support this hypothesis.

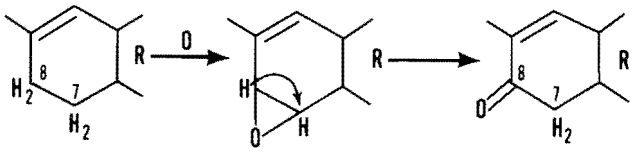

Figure 9. *Mechanism of epoxidation at carbon-8.*

The third route to trichothecin involves hydroxylation of trichodermol at C-8 followed by oxidation. Incubation of 12,13-epoxy-4β,8α-dihydroxytrichothec-9-ene with *T. roseum* results in formation of trichothecolone with a 6.6% incorporation (*14*). Trichothecolone, which co-occurs with trichothecin in *T. roseum*, was converted into trichothecin by the fungus in 27% yield. Therefore, esterification is probably one of the last stages of biosynthesis.

In summary, the biosynthetic pathway as currently known for trichothecin involves the following sequence:

3 mevalonic acids → farnesyl pyrophosphate → trichodiene → trichodiol → 12,13-epoxytrichothec-9-ene → trichodermol → 12,13-epoxy-4β,8α-dihydroxytrichothec-9-ene → trichothecolone → trichothecin (Fig. 10).

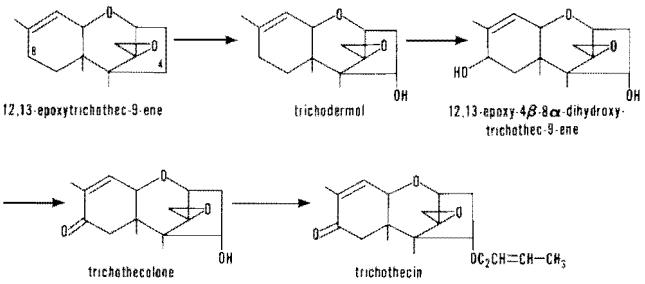

Figure 10. *Biosynthesis of trichothecin.*

The biosynthesis of the verrucarins and roridins have been investigated extensively at Tamm's laboratory in Basel and his publications should be consulted for detail (*4,5,8,21-23*). The di- and triester macrocyclic moieties of the more complex verrucarins and roridins have been shown by these investigators to be derived from mevalonic acid. Verrucarol appears to be the sesquiterpene common to both the verrucarins and roridins and is obtained on base hydrolysis of the parent compounds (*21,22*) (Fig. 11).

What has been outlined up to this point presents a relatively satisfying explanation for the general biosynthesis of the 12,13-epoxytrichothecenes. However, a paper published recently by Breitenstein and Tamm (*7*) has raised a possible question. These investigators isolated a new metabolite in low yield, verrucarin K, from *Myrothecium verrucaria* (Fig. 12). This compound represents the first natural trichothecene derivative lacking the 12,13-epoxy group, this moiety being replaced by an exocyclic double bond.

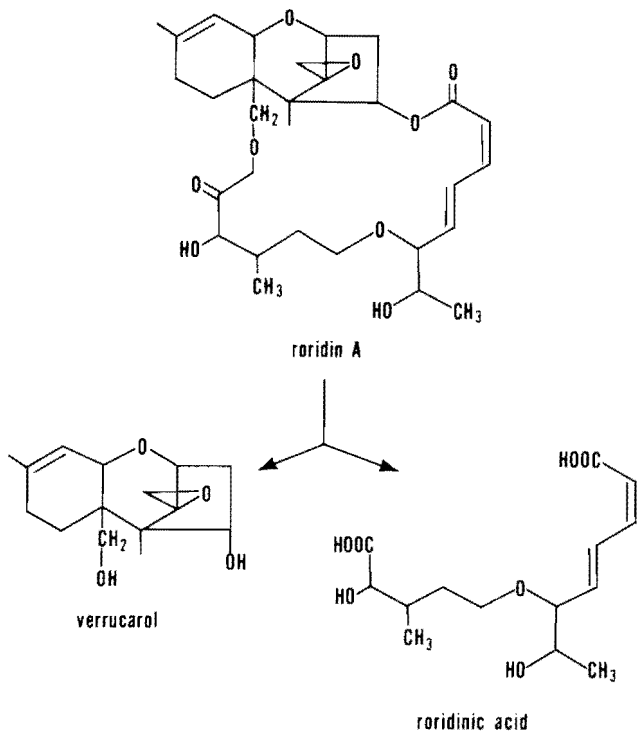

Figure 11. *Structure of roridin A.*

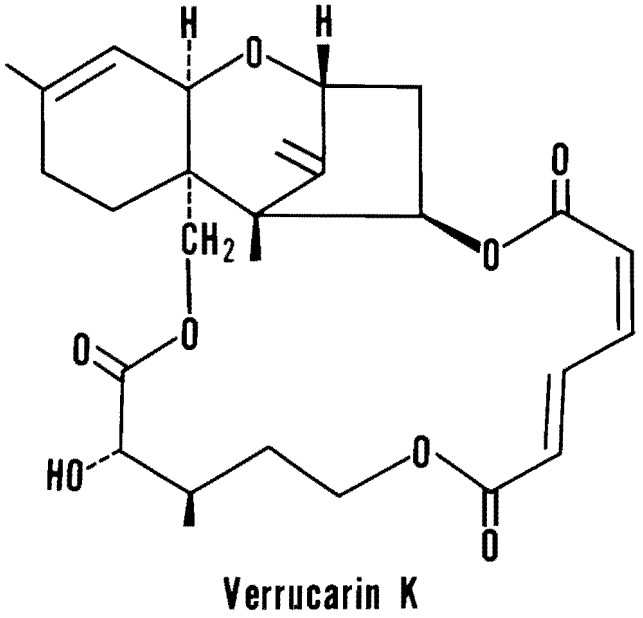

Figure 12. *Structure of verrucarin K.*

If the biogenesis of verrucarin K involves trichodermol as an intermediate as is postulated for all known 12,13-epoxytrichothecenes, then reductive removal of the preformed epoxy function would be required at a later stage. Tamm presented no experimental evidence to preclude this possibility. However, an alternative to the reaction sequence previously shown can be postulated by the direct cyclization of an intermediate, trichodiol, shown in a previous figure to the trichotheca-9,12-diene system (Fig. 13).

A compound very similar to trichodiol (hydroxydienone) was an actual key intermediate for the cyclization

reaction in a biomimetic total synthesis by Masuoka and Kamikawa (*19*) (Fig. 14).

One can also postulate, under the circumstances, that there is possibly more than one road to Rome.

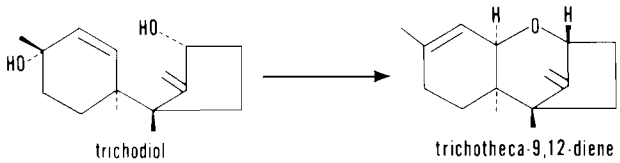

Figure 13. *Direct cyclization of trichodiol.*

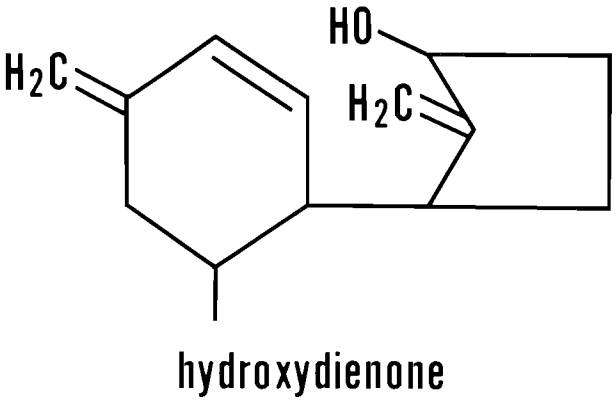

Figure 14. *Structure of hydroxydienone.*

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