Qualitative, Quantitative and Technological Aspects of the Trichothecene Mycotoxins

A. PETER SNYDER

AMCCOM, Chemical Research, Development and Engineering Center, SMCCR-RSL, Aberdeen Proving Ground, Maryland 21010-5423

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ABSTRACT

Trichothecene mycotoxins pose a natural threat to plants, foodstuffs, animals and humans. Recently, strong implications regarding artificially induced trichothecene threats to humans in various parts of the world have come to the attention of the general public. This has spawned renewed interest and scientific research into the various properties of the toxins. The trichothecenes display orders of magnitude differences in toxicity levels depending upon the test subject and mode of administration. Potentially more sensitive and specific analytical characterization techniques and convenient, milder and faster organic decontamination reaction schemes exist in comparison to established methods. This review attempts to supply a concise information source as an aid to investigators faced with problems of trichothecene detection, analysis, and decontamination.

During the past century, world-wide documented outbreaks of massive intoxication and death, resulting from trichothecene-infected foodstuffs, have been documented (Table 1). This sampling of the literature presents evidence that the trichothecene-producing fungi are pathogens found in various plants and in the fruit that they bear. These compounds are a class of mycotoxins (from the Greek word ‘‘mykes’’ meaning ‘‘fungus’’ and the Latin word ‘‘toxicum’’ meaning ‘‘poison’’) and are a threat to the well-being of humans and animals. Whenever outbreaks of pathogenic bacteria, viruses, or fungi occur or when unusually large amounts of the toxins themselves are found in nature, whether of natural or artificial origin, a careful and complete investigation is warranted. In the last decade, the possibility of artificially induced mycotoxin threats to humans in various parts of the world has come to the attention of the general public (4, 33, 34, 151, 190).

The mycotoxins chosen for concentrated review in this report are the trichothecenes, because most of the work performed to date (with the possible exception of the aflatoxins) has been associated with the trichothecenes in the agricultural, chemical, biological, and toxicological fields. Extensive research has been conducted over the past two decades on these naturally occurring compounds concerning their toxic properties and possible carcinogenic and therapeutic potential. The scientific community continues to address these problems to control the occurrence of the toxins in human and animal food sources, including the possibility of their long-term exposure in foodstuffs (96, 144, 195). This literature review attempts to supply a concise information source as an aid to investigators faced with problems of trichothecene detection, analysis, and decontamination.

PHYSICAL CHARACTERISTICS AND TOXICITIES OF TRICHOTHECENES

The trichothecenes encompass a broad group of naturally occurring compounds, and they are produced by the fungal genera Fusarium, Myrothecium, Stachybotrys, Cephalosporium, and Verticimonomosporium. The verrucarvin group (14), initially named glutinosin, were the first trichothecenes to be isolated (1946), and it was more than a decade later (1960) before isolation of the next trichothecene compound, diacetoxyscirpenol (49). In 1967, the scirpene compounds nivalenol and fusarenon-X were isolated from Fusarium nivale by Tatsuno et al. (167) and Ueno et al. (183). In the late 1960s, a toxic compound was isolated from Fusarium tricinctum-infected corn (48). It was analyzed as T-2 toxin (8) and induced edema and intradermal hemorrhage on rat skin. The over 47 naturally occurring trichothecenes as a group have a number of elements in common (155). The most obvious is the trichothecan ring system. In fungi, the trichothecenes are derived from a cyclization of the precursor molecule farnesyl pyrophosphate, involving a 1,2-dimethyl double migration (79). They belong to a family of sesquiterpenoids (i.e., one and one-half terpene units in the A-ring of the trichothecenes) (Fig. 1) with ester and alcohol functions residing on the periphery of the toxin molecules. They also have a carbon-9,10 double bond and an epoxy group at carbon-12,13, hence, they are usually described as 12,13 epoxytrichothecenes.

Various modes of trichothecene classification are employed in the literature. Figures 1 through 4 present the toxins and their chemical-structural relationships (123). The trichothecenes are classified into the arbitrary groups;
A, B, C, and D. The group A trichothecenes (Fig. 1a, b) have a hydroxy or acetoxy function primarily at R₁, R₂, R₃, and R₅. Figure 1b depicts the stereochemical structure of a typical group A trichothecene, diacetoxyscirpenol, with the α-face residing above the molecular plane and the β-face below (2,152). Note that the six-membered B ring has a chair conformation, and the five-membered C ring adopts an envelope form with carbon-12 as the flap. Also, the R₂ acetyl carbonyl oxygen hydrogen-bonds (dashed line in Fig. 1b) with the α-hydrogen of carbon-3 forming a six-membered ring. The group B trichothecenes (Fig. 2) contain a carbonyl at R₅ (carbon-8) in addition to the group A functions at R₁, R₂, R₃, and R₄. The macrocyclic trichothecenes (Fig. 3) comprise group C, attaching their broad-based, carbon-oxygen ring system to the R₂ and R₃ positions of the basic trichothecene structure. Figure 3 portrays examples of the more than 30 known macrocyclic members. Crotoxin (Fig. 4), a nonmacrocyclic, diepoxylvirginol is in a class by itself, group D. Groups A, B, and D comprise the simple ester and alcoholic trichothecenes while group C encompasses the macrocyclic compounds.

Trichothecene structure and absolute configuration determinations were first resolved for trichothecolone, trichodermol, and verrucarol (162). The primary analytical techniques were X-ray diffraction of the p-bromoben...
zoate of trichodermol (1,2) and nuclear magnetic resonance (NMR) spectroscopy. By inference, the three-dimensional structures of the other simple trichothecenes were assumed (162). The determination of three-di-

![Figure 1. Group A trichothecenes (T-2 type). (a) (top) The basic structure of the T-2 type trichothecene molecule with a compendium of the majority of the group A trichothecenes. (b) (bottom) The stereochemical structure of diacetoxyscirpenol.](image)

<table>
<thead>
<tr>
<th>Trichothecene</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>$R_5$</th>
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<tr>
<td>Basic Trichothecene</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<tr>
<td>Trichodermol (roridin C)</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<td>H</td>
<td>OAc$^a$</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<td>OH</td>
<td>OH</td>
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<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
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<tr>
<td>Monoacetoxyscirpenol (MAS)</td>
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<td>OH</td>
<td>OAc</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Diacetoxyscirpenol (anguidine) (DAS)</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>7-Hydroxy DAS</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>OH</td>
<td>H</td>
</tr>
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<td>Calonectrin</td>
<td>OAc</td>
<td>H</td>
<td>OAc</td>
<td>H</td>
<td>H</td>
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<tr>
<td>15-Diacetylcalonectrin</td>
<td>OAc</td>
<td>H</td>
<td>OH</td>
<td>H</td>
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<tr>
<td>Dihydroxy trichothecene</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>T-2 tetraol</td>
<td>OH</td>
<td>OAc</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Neosolaniol (solaniol)</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Monoacetyleneosolaniol</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>H</td>
<td>OAc</td>
</tr>
<tr>
<td>7,8-Dihydroxy DAS</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>H</td>
<td>b</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>OH</td>
<td>OAc</td>
<td>OAc</td>
<td>H</td>
<td>b</td>
</tr>
<tr>
<td>Acetyl T-2 toxin</td>
<td>OAc</td>
<td>OAc</td>
<td>OAc</td>
<td>H</td>
<td>b</td>
</tr>
</tbody>
</table>

$^a$ OAc, acetate

$^b$ $-O-C\text{CH}_2\text{CH(Me)}_2$, isovalerate

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The trichothecenes in general are colorless, crystalline, optically active, and stable in the solid state. Presented in Table 2 are some physical properties of selected trichothecenes. The trichothecenes listed can be stored at room temperature for years or heated at 100°C for up to 1 h with no loss of activity. A concise compendium of the trichothecenes and other mycotoxin physical constants and spectral data can be found in the handbook by Cole and Cox (23).

Occurrence, pathology, and toxicity of trichothecenes

Production of these toxins by fungi is somewhat diverse. Each of the species produces some or all of the toxins, as listed in Table 3, as their natural metabolites. The fungi themselves are found all over the world in agricultural products and plants. Table 4 lists the results of studies of toxin concentrations in different agricultural samples. It is interesting to note that the triepoxy trichothecene baccharin, isolated from Baccharis megapotamica Spreng (Asteraceae), was the first and only trichothecene to have been isolated from higher plants without the fungus responsible for its production (86). The yield of the macrocyclic trichothecene was 0.02% (w/w) from the dried plant which is high enough to kill tomatoes, peppers, and artichokes (73).

Tables 1 and 4 present evidence that mycotoxin infestation occurs naturally in basic foodstuffs, e.g., oats, hay, rye, and corn. These foodstuffs, in turn, are ingested by farm animals and humans, and if toxin elimination methods (chemical or physical) are not undertaken, dire consequences can result. Various symptoms range from headache, nausea, and vomiting to death. Table 5 summarizes a few of the experiments that were undertaken to assess the effects of different trichothecenes and their fungal sources by reproducing the naturally-occurring illnesses in different laboratory animals. Their acute pathology resembles that caused by radiation (radiomimetic agents) or of alkylating agents such as nitrogen mustard (142). No obvious symptoms relating to carcinogenic effects were observed. Further corroborating evidence of a noncarcinogenic effect resulted by using T-2 toxin in long-term, low-dose feedings in rainbow trout (96). A review by Hayes (55) concluded that the trichothecenes lack mutagenic activity to bacteria and, with one exception (vide infra), fungi and yeast, with or without metabolic activation using S-9 microsomal fraction. This observation was also supported by Talmage et al. (160) in a recent, extensive review of the trichothecene mycotoxin literature. One exception is that fusarenon-X and nivalenol have been observed to induce mutations in the yeast organism Saccharomyces cerevisiae (184). However, Ueno et al. (177) found that with the supernatant S-9 fraction, 100-500 µg/agar plate of fusarenon-X produced no mutagenic response with the microorganism Salmonella typhimurium TA100, while Sugimura et al. (156) and Ohtsubo and Saito (120) report positive mutagenic observations with the same system, the latter reporting toxin doses of 500-1000 µg/plate.

Relative and absolute measures of toxic doses of the trichothecene poisons were given attention. Bamburg and Strong (10) and Ueno et al. (174) performed skin irritability experiments on the shaved backs of guinea pigs, mice, and rabbits with the result that the group A toxins were 10 times as active as the group B toxins. Antiproliferative activity (Tetrahymena pyriformis GL) studies indicated that in the group A toxins, T-2 and DAS are 10 times as effective as HT-2 and neosolaniol. Collectively, the group A toxins are 50 times as toxic as the group B toxins (108,186). In the cytotoxicity experiments (Table 6) utilizing the inhibition of protein synthesis assay in rabbit reticulocytes (vide infra), the verrucarins and roridins (group C) are three times as potent as the group A toxins, and the latter group is approximately 10 times as toxic as the group B compounds (179,181). These results suggest that since group A is more toxic than group B, greater toxicity is achieved when the R3, R4, and R4 functions are esterified. Within group A, the reduced toxicity of neosolaniol in all three experimental assays can be ascribed to the lack of the methylbutyryl group which T-2 toxin possesses. Increased toxicity is realized in the group B compounds by acetylating the side groups of nivalenol to produce the toxins fusarenon-X and DAN. Hence, increasing the lipophilicity upon esterification results in a concomitant increase in toxicity. This is reinforced by work with cell cultures (52) and chick embryos (69). This trend is observed in groups A and B, both collectively and separately (Table 6).

Inspection of the results of intraperitoneal (ip) studies presented in Table 7 indicates that the acute toxicity of the macrocyclic compounds is greater than that observed in the group A and B trichothecenes, and slightly higher oral toxicity (22) exists over an ip administration with most of the trichothecenes. However, two further noteworthy points (184,197) which are in conflict with the data in Table 6 are: (a) the hydroxyl group at carbon-4 (R3) of the trichothecene nivalenol imparts greater or similar toxicity in comparison to its esterified forms and (b) within each of the two toxin series in group B, less lipophilicity generally imparts greater toxicity.
Figure 3. The basic structure and diagrams of representative group C macrocyclic trichothecenes.
Figure 4. The structure of the group D trichothecene crotocin.

Lindenfelser et al. (93) presented evidence of synergism in mixtures of different mycotoxins. Toxins were introduced into female mice (ip). A graph of similar parameters (isobologram) was constructed to depict the response of T-2 toxin when combined with aflatoxin B (Fig. 5). The dashed line shows what LD<sub>50</sub> values would be expected, assuming an additive response. The solid curve represents the experimentally observed synergistic effect that occurs with the two mycotoxins in which it was observed that one toxin greatly enhanced the other's response. Similar effects were observed with ochratoxin A and penicillic acid (94). An interesting effect was observed by Wogan et al. (193) when the non-trichothecene mycotoxins rubratoxin B and aflatoxin B<sub>1</sub> were examined with rats. One group of rats was fed three times a week with rubratoxin B, another group was fed aflatoxin B<sub>1</sub> and a third group was fed the two mycotoxins simultaneously. No fatalities were observed when the animals were fed only one mycotoxin. A high mortality rate resulted from simultaneous feeding of the two mycotoxins. An additive as opposed to a synergistic effect was noticed by Ohtsubo et al. (182) when mice were fed fusarenon-X and penicillic acid simultaneously.

### ISOLATION, PURIFICATION, AND DETECTION OF TRICHOTHECENES

A phenomenon that is common to the toxin-producing fungi exists in the difference in the storage conditions that can cause infection of foodstuffs. Depending on the environmental and nutrient conditions, various amounts of toxin can be produced in addition to the generation of different types of toxins. For example, in the laboratory, <i>F. nivale</i> produces fusarenon-X on a peptone liquid medium while on a moistened rice medium, greater amounts of nivalenol are produced (776). <i>F. tricinctum</i>

### TABLE 2. Physical Properties of Selected Trichothecenes

<table>
<thead>
<tr>
<th>Trichothecene</th>
<th>State or shape</th>
<th>Recrystallizing medium</th>
<th>Melting point, °C</th>
<th>[d]&lt;sub&gt;D&lt;/sub&gt; &lt;sup&gt;a&lt;/sup&gt;</th>
<th>e&lt;sub&gt;λ&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; solvent&lt;sub&gt;λ&lt;sub&gt;max&lt;/sub&gt;&lt;/sub&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON</td>
<td>Needles</td>
<td>Ethyl acetate and petroleum ether</td>
<td>151-153</td>
<td>+6.35&lt;sub&gt;D&lt;/sub&gt;</td>
<td>4,500&lt;sub&gt;EtOH&lt;/sub&gt; 218</td>
<td>192,196</td>
</tr>
<tr>
<td>DON triacetate</td>
<td>Colorless needles</td>
<td>Ethyl acetate and petroleum ether</td>
<td>155-157</td>
<td></td>
<td>192,196</td>
<td></td>
</tr>
<tr>
<td>Nivalenol</td>
<td>Crystals</td>
<td>Methanol</td>
<td>222-223</td>
<td>+21.5&lt;sub&gt;EtOH&lt;/sub&gt;</td>
<td>7,500&lt;sub&gt;MeOH&lt;/sub&gt; 218</td>
<td>106,166</td>
</tr>
<tr>
<td>DON monoacetate</td>
<td>Either and n-pentane</td>
<td></td>
<td>185.5-186</td>
<td>+430&lt;sub&gt;MeOH&lt;/sub&gt;</td>
<td>5,900&lt;sub&gt;EtOH&lt;/sub&gt; 219</td>
<td>11,192,196</td>
</tr>
<tr>
<td>Diacetyl DON</td>
<td>Ethanol</td>
<td></td>
<td>119-120</td>
<td></td>
<td>196</td>
<td></td>
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<tr>
<td>T-2 toxin</td>
<td>White needles</td>
<td>Benzene</td>
<td>151-152</td>
<td>+15&lt;sub&gt;EtOH&lt;/sub&gt;</td>
<td>8,75,76</td>
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<td>Satratoxin G</td>
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<td></td>
<td>167-170</td>
<td></td>
<td>36,37</td>
<td></td>
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<tr>
<td>Satratoxin H</td>
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<td></td>
<td>162-166</td>
<td></td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>Yellow oil</td>
<td></td>
<td>119-120</td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Fusarenon-X</td>
<td>Hexagonal</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt; and n-pentane</td>
<td>91-92</td>
<td>+58&lt;sub&gt;MeOH&lt;/sub&gt;</td>
<td>6,500&lt;sub&gt;MeOH&lt;/sub&gt; 220</td>
<td>161,185</td>
</tr>
<tr>
<td>DAN</td>
<td>Crystals</td>
<td>Acetone and hexane</td>
<td>135-136</td>
<td>+64.3&lt;sub&gt;EtOH&lt;/sub&gt;</td>
<td>6,200&lt;sub&gt;MeOH&lt;/sub&gt; 220</td>
<td>44,165</td>
</tr>
<tr>
<td>Neosolaniol</td>
<td>Crystals</td>
<td>Ethyl acetate and n-hexane</td>
<td>171-172</td>
<td>c</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>DAS</td>
<td>Crystals</td>
<td>Ether</td>
<td>162-164</td>
<td>c</td>
<td>28,40</td>
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<tr>
<td>MAS</td>
<td>Crystals</td>
<td>Isooctane and ethyl acetate</td>
<td>172-173</td>
<td>c</td>
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<tr>
<td>Baccharin</td>
<td>Crystals</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt; and MeOH</td>
<td>200-230</td>
<td>+41.5&lt;sub&gt;CHCl&lt;sub&gt;3&lt;/sub&gt;&lt;/sub&gt;</td>
<td>18,700&lt;sub&gt;EtOH&lt;/sub&gt; 259</td>
<td>86</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> The specific optical rotation at 25°C using the sodium D line
<sup>b</sup> The molar extinction coefficient of the compound as measured at the listed wavelength in the given solvent
<sup>c</sup> End absorption, <230 nm
TABLE 3. Trichothecene-Producing Fungi\textsuperscript{a}

<table>
<thead>
<tr>
<th>Trichothecene and source</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A and B</td>
<td>C</td>
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<tr>
<td>T-2 toxin</td>
<td>Nivalenol</td>
<td>DAS</td>
<td></td>
<td>Roridins</td>
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<tr>
<td>HT-2 toxin\textsuperscript{b}</td>
<td>Fusarenon-X</td>
<td>DAN</td>
<td>7-Hydroxy DAS</td>
<td>Verrucarsins</td>
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<tr>
<td>DAS</td>
<td>DAN</td>
<td>7,8-Hydroxy DAS</td>
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<td></td>
</tr>
<tr>
<td>Neosolaniol</td>
<td>DON</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fungus

- *Fusarium tricinctum*
- *F. roseum var. graminearum*
- *F. sporotrichioides*
- *F. poae*
- *F. solani*

- *F. nivale*
- *F. roseum*
- *F. equiseti*
- *F. scirpi*
- *Myrothecium verrucaria*
- *M. roridum*

- *Stachybotrys atra*
- *S. alternans*
- *Dendrodochium toxicum*

\textsuperscript{a} References 8,13,19,70,113,123,180

\textsuperscript{b} A metabolite

TABLE 4. Trichothecene Contamination Levels Found in Agricultural Products

<table>
<thead>
<tr>
<th>Trichothecene</th>
<th>Sample</th>
<th>Place</th>
<th>Concentration</th>
<th>Reference</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>mg/kg</td>
<td>ppm</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>Corn</td>
<td>Wisconsin</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>DON</td>
<td>Corn</td>
<td>Ohio</td>
<td>3</td>
<td>188</td>
</tr>
<tr>
<td>DON</td>
<td>Corn</td>
<td>Illinois</td>
<td>8</td>
<td>71</td>
</tr>
<tr>
<td>DON</td>
<td>Corn</td>
<td>Indiana</td>
<td>8</td>
<td>196</td>
</tr>
<tr>
<td>DON</td>
<td>Barley</td>
<td>Kagawa, Japan</td>
<td>4</td>
<td>196</td>
</tr>
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</table>

TABLE 5. The Effects of Trichothecenes/Fungi on Laboratory Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Trichothecene</th>
<th>Fungus</th>
<th>Mode of application</th>
<th>Diagnosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats, mice</td>
<td>T-2 toxin</td>
<td><em>F. nivale</em></td>
<td>Oral – feed</td>
<td>Atrophy of thymus, spleen, bone marrow, and testicles; bronchopneumonia</td>
<td>141,143</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>F. graminearum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarenon-X</td>
<td></td>
<td>Oral</td>
<td>Same as above</td>
<td>141,143</td>
</tr>
<tr>
<td>Rats</td>
<td>T-2 toxin</td>
<td></td>
<td>Oral – feed</td>
<td>Lesions and extreme papillary growth inside the gastrointestinal tract</td>
<td>93,173</td>
</tr>
</tbody>
</table>
TABLE 6. Toxicities of Trichothecenes

<table>
<thead>
<tr>
<th>Assay</th>
<th>Group</th>
<th>Number of lipophilic R-functions</th>
<th>Trichothecenes</th>
<th>Guinea pigs</th>
<th>Mice</th>
<th>Rabbits</th>
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</thead>
<tbody>
<tr>
<td>Skin necrotization</td>
<td>A</td>
<td>3,2</td>
<td>T-2, HT-2</td>
<td>0.2</td>
<td>1</td>
<td>–</td>
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<tr>
<td></td>
<td>B</td>
<td>2,2</td>
<td>DAS, neosolaniol</td>
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<td></td>
<td></td>
<td>1,2</td>
<td>Fusarenon-X, DAN</td>
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<td>10</td>
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<td>DON monoacetate</td>
<td>29</td>
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<td>Cytotoxicity</td>
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<td>Verrucarin A, roridin A</td>
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<td>A</td>
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<td>DAN, fusarenon-X, trichothecin</td>
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<td>0</td>
<td>Trichothecolone</td>
<td>20</td>
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</table>

a Minimum effective dose
b Dose for 50% inhibition of protozoal multiplication
c Dose for 50% inhibition in the “whole cell” assay system

Purification of trichothecenes

The trichothecenes can be classified as belonging to either group I or group II depending on their solubility in various solvents. Group I (Fig. 6a) toxins are efficiently extracted from mixtures in aprotic solvents such as chloroform, methylene chloride, ethyl acetate, diethyl ether, and acetone. Group II toxins (Fig. 6b), due to their greater abundance of polar side groups, are soluble in such solvents as ethanol, methanol, water, aqueous methanol, and aqueous acetonitrile. It was found that the solvent system of choice for the extraction of a mixture of group I and group II compounds is ethyl acetate and acetonitrile (109). The drawback to this procedure is the extensive purification needed to remove the ethyl acetate from the group I fraction. Separation techniques for the trichothecenes from their crude extracts consist of different liquid/liquid or solid/liquid partitions. Some liquid/liquid systems in use (66) are a 1:1 (v/v) solution of 50% MeOH/ethyl acetate-CHCl₃ and a solution of aqueous MeOH/petroleum ether (60-70°C). Both systems are currently used in the extraction of T-2 toxin. A mixture of H₂SO₄ (0.8 N)/ethyl acetate is used for DAS and a 1:1 (v/v) solution of acetonitrile/petroleum ether (60-70°C) can be conveniently used in the separation of MAS, DAS, or T-2 toxin (103,124). Other frequently used extraction solvents and partition systems for trichothecene...
TABLE 7. Intraperitoneal Toxicities

<table>
<thead>
<tr>
<th>Group</th>
<th>Trichothecene</th>
<th>Acetate location</th>
<th>LD50, mg/kg</th>
<th>Intraperitoneal&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Oral&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Verrucarins, roridins</td>
<td>–</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>T-2 toxin</td>
<td>–</td>
<td>5.2</td>
<td>5.0</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>HT-2 toxin</td>
<td>–</td>
<td>9.0</td>
<td>7.2</td>
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<tr>
<td></td>
<td>Neosolaniol</td>
<td>–</td>
<td>14.5</td>
<td>25.0</td>
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<td>DAS</td>
<td>–</td>
<td>23.0</td>
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<tr>
<td>B</td>
<td>DON</td>
<td>–</td>
<td>70</td>
<td>46</td>
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<tr>
<td></td>
<td>3-Acetyl DON</td>
<td>R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>49</td>
<td>34</td>
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<td>3,15-Acetyl DON</td>
<td>R&lt;sub&gt;1&lt;/sub&gt;,R&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>4-Acetyl nivalenol</td>
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<td></td>
<td>(fusarenon-X)</td>
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<tr>
<td></td>
<td>4,15-DAN</td>
<td>R&lt;sub&gt;2&lt;/sub&gt;,R&lt;sub&gt;3&lt;/sub&gt;</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Dose for 50% of the mice to succumb.

<sup>b</sup> Dose for 50% of the 1-day-old broiler chicks to succumb (group A) and dose for 50% of the mice to succumb (group B).

Figure 5. Isobologram showing synergistic effects of mycotoxins. The plot depicts the various combined doses of two mycotoxins in female mice (mg/kg). The dashed line assumes an additive response. Redrawn from reference 93.
The absorbance of the resulting solution was measured at 583 nm, and fusarenon-X and T-2 toxin could be determined in the 1 to 20 μg range in a 20 μL sample. To circumvent spectral limitations one routinely turns to several chromatographic determinations. The simplest quantitative analysis is the TLC technique. The resulting spots are made visible by exposing the plate either to iodine vapor or concentrated sulfuric acid spray, heating to approximately 105°C, and noting the blue fluorescence under longwave UV light (ca. 356 nm). By spraying the plates with either a concentrated sulfuric acid or p-anisaldehyde solution (150), the trichothecenes exhibit characteristic colors. The group B toxins, because of their α,β-eno system, produce a nonfluorescent brown spot (180). Naoi et al. (111) has found that by spraying a TLC plate with 50% AlCl₃ and heating, fusarenon-X reacts with ZrO(NO₃)₂ in the presence of ethylenediamine to produce an adduct in which 25 ppb can be detected by fluorescence.

Recently three more sensitive TLC techniques have been developed. The first procedure utilizes the fact that the pyridine nitrogen of the compound 4-(p-nitrobenzyl) pyridine (NBP) attacks epoxide moieties and conjugates with the carbon-12 portion of the trichothecene epoxide (159). The resulting blue spot observed on the plate is then analyzed by spectrophotodensitometry. Linear standard curves of group A and B trichothecenes are attained at levels of 0.05 to 10 μg/spot. Positive results are realized with this procedure as opposed to negative results from the application of reagents such as Na₂S₂O₃, MgCl₂, HCl, and picric acid. Its specificity is defined by negative NBP results with trichothecene-like compounds lacking the epoxide ring. The second procedure, a sensitive and specific fluorometric one, is based on alkylation using the reagent nicotinamide (112,137). Briefly, the epoxide-containing compound is added to a solution of nicotinamide, a ketone (e.g., acetophenone) and alcoholic KOH. The fluorescent species is subsequently generated with the addition of formic acid. It is approximately 100 times more sensitive than the NBP method in that 0.1 to 2.0 ng of epoxy compounds can be detected. Another desirable property of this technique is that it is performed under ambient conditions versus the requisite 0.5 h of heating at 150°C in the NBP analysis. The third technique (198), continuous multiple-development, high-performance TLC (HPTLC), provides an extremely sensitive and yet rapid screening method without prior sample derivatization. Thirteen UV-absorbing, short wavelength VIS fluorscencing mycotoxin compounds (not including the trichothecenes) were successfully separated by Lee et al. (91). Detection limits were in the nanogram and picogram range for the UV-VIS and fluorescence detection methods, respectively. For nonfluorescing species (e.g., the trichothecenes) various sprays (vide supra) can be used in certain plate development stages to induce sample fluorescence and therefore detection.

NMR and IR absorption analyses are routinely used in the initial identification and structure determination stages of trichothecene analysis. An exhaustive collection of NMR and IR spectra can be found in the handbook by Cole and Cox (23).
Gas-liquid chromatography (GLC) is routinely utilized in the identification of mixtures of trichothecenes. Derivatization of the hydroxyl functions is vital so as to impart sample volatility, which permits separation and identification of the toxins in a mixture. Sample analyses are routinely performed using a flame ionization detector (FID). A few examples of derivatization and GLC analyses follow. Various agents were reacted with a mixture of N-trimethylsilylimidazole (SIM) and trimethylchlorosilane (TMCS) in pyridine which resulted in the complete silylation of the group II toxins. Satisfactory retention times resulted in chromatographic peaks of good resolution. Incomplete silylation of group II toxins occurred with the use of bis(trimethylsilyl)acetamide (BSA) or hexamethyldisilazane (164). Mirocha et al. (103) found that a 3:2:3 mixture of BSA, TMCS, and SIM ensured complete silylation of DON in the absence of solvent. Ohta et al. (178) accomplished the separation of a mixture of seven group I and four group II toxins by the judicious choice of proportionate amounts of silylating agents and application of the derivatized toxins onto a column packed with 1.5% OV-17. The silylation reagent, composed of a 5:1:5 mixture of BSA, TMCS, and SIM (compare with the data of Mirocha et al. (103)) completely derivatized the toxins in 5 min at room temperature. The group I toxins themselves can be analyzed (124) by derivatizing them with N-methyl-bis(trifluoroacetamide), (CF3CO)2NCH3, provided that interfering compounds from the original extract are satisfactorily removed.

Another GLC method utilizes the electron capture detector (ECD). A number of research groups (82,87,135,149) have used the GLC/ECD technique in the detection of the trichothecenes. By using either the SIM/TMCS or heptafluorobutyric acid derivatizing reagents, detection limits of approximately 5 pg and 400 pg of a number of the group II and group I toxins, respectively, have been obtained. In comparison, by derivatizing the sample with more efficient electrophoric silyl functions and using the ECD mode, greater sensitivity is observed than that of a sample derivatization using an alkyl-silyl function and flame ionization detection. Various research groups using this ECD technique have reported picogram to femtogram limits of sample detection. Poole et al. (18,104,105,127,131,199) have prepared flophemesyl (pentfluoroanilypentafluoroacetamide) (CF3CO)2NCH3, provided that interfering compounds from the original extract are satisfactorily removed.

Both GC/MS techniques have been expanded to multiplex-stage, MS analyses or mass spectrometry/mass spectrometry (MS/MS)(24). The strength of this technique lies in the fact that a greatly increased signal-to-noise ratio, despite overall loss of signal strength, is achieved from one MS stage to the next by a reduction in chemical noise. Most applications of this technique involve chemical ionization, mass selection of a parent ion, collisionally induced fragmentation and subsequent recording of a daughter ion MS/MS spectrum. Very low detection limits have resulted for various compounds. For example, 2,3,7,8-tetrachlorodibenzo-p-dioxin, despite the presence of an excess of the interferential compound polychlorinated biphenyl, has been detected at less than 50 pg.

Various chromatographic techniques coupled with sample-derivatized, fluorescence monitoring methods have been reported in the literature that could prove quite profitable in trichothecene detection and identification. One technique involves the use of high performance liquid chromatography (HPLC) (57,77,78) or TLC (47) coupled and the inherent hydroxyl groups in the toxins, the flophemesyl-GLC/ECD combination appears to have a potential application in trichothecene detection. Greater specificity could be attained by using either the substituted benzeneboronic acids (BB) (129,130,153,199) or ethylphosphonothioic dichloride (EPTD) (128) as derivatizing agents. They both react with bifunctional compounds wherein both functions are either OH, NH2, or COOH separated by up to two methylene units. Picogram detection is realized with BB derivatization, and by using a phosphorus detector with the EPTD reagent instead of a thermal electron detector, femtogram quantities can be attained. Using GLC/ECD, Corkill et al. (25) have presented experimental evidence of approximately 10-17 g (90 attograms) detection with a signal-to-noise ratio of 2 of a new compound that has the potential to act as a derivatizing reagent. The compound, N,N-dipentafluorooxyalkylpentfluoroaniline (DFPA) is essentially a modified version of N-methyl-bis(trifluoroacetamide) with flophemesyl features.

Even though GLC/FID is a sensitive tool, it was found by Mirocha et al. (102) that three samples of feed analyzed in different laboratories resulted in negative T-2 toxin diagnosis using gas chromatography/mass spectrometry (GC/MS) while a GLC technique gave false positive findings of the toxins. This usually occurs at the limit of detection level and is compounded by the problem of a moderate amount of interfering lipid substances that co-chromatograph with the toxin from the sample. Because a normal GC/MS analysis can circumvent this problem, it provides more reliable information with greater sensitivity. The selected ion monitoring spectrometry (103) (SIMS) GC/MS technique produces an even greater resolution of the trichothecene derivatives because it can be used in detecting single or multiple ion signals. Table 8 presents a comparison of the resolution that is obtained using different methods of trichothecene isolation and identification.
TABLE 8. Detection Limits of Trichothecenes

<table>
<thead>
<tr>
<th>Trichothecene</th>
<th>GC/MS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GLC&lt;sup&gt;a&lt;/sup&gt;/FID, μg/μl injection</th>
<th>TLC&lt;sup&gt;b&lt;/sup&gt; μg/spot</th>
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<tr>
<td>DON</td>
<td>0.007</td>
<td>0.02</td>
<td>0.025</td>
</tr>
<tr>
<td>MAS</td>
<td>0.015</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>DAS</td>
<td>0.009</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Neosolaniol</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>0.02</td>
<td>0.04</td>
<td>0.085</td>
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</table>

<sup>a</sup> Data presented from TMS ether derivatives of the toxins

<sup>b</sup> H<sub>2</sub>S<sub>4</sub> spray

with video fluorimetry (VF). Video fluorimetry employs a novel irradiation geometry and image detector to simultaneously collect excitation and emission spectra of fluorescent compounds. Along with the retention time, the excitation and emission matrix can subsequently be treated to resolve known and unknown spectrally overlapping, fluorescent compounds for further analyses. Coupling the HPLC-separated, nicotinamide-derivatized sample with VF, a potentially sensitive and specific technique for epoxy-containing toxins exists. A second technique utilizes TLC (47). The intensities of the fluorescent spots can be monitored with a silicon intensified target vidicon camera by irradiating the plate with UV radiation or with a laser for even greater sensitivity. Picogram quantities of the sample can be detected. In a second TLC format, an emission spectrum from each position along the elution axis of the plate is obtained, and this results in spectral fingerprinting and R<sub>f</sub> information of the entire spotted sample.

CHEMICAL REACTIONS OF TRICHOTHECENES

A number of chemical reactions have been reported in the literature using the trichothecenes, including oxidation, reduction, catalytic hydrogenation, and acid and base reactions. Various chemical reactions are listed in Figure 7a-d by reaction type, reagent used, and a representative group A or B trichothecene. The oxidation reaction utilizing perbenzoic acid [Fig. 7a(10)] is interesting in that the α-epimer (epoxide ring facing away from the viewer) is more stable than the β-epimer (epoxide ring facing toward the viewer). The latter undergoes intramolecular nucleophilic substitution to form another six-membered ring. Various other ring closure reactions are presented, destroying the 12,13-epoxy function in the process, thereby eliminating toxicity. Figures 7b(1) and 7c present reactions that destroy the epoxide ring and form the apotrichothecene ring system that is devoid of toxicity. These reactions do not generate another ring which occurs in the case of ring closure reactions in Fig. 7d (3, 4). The reactions portrayed in Fig. 7a(5, 6) afford the α-stereochemical substitution of a hydroxyl group since that particular face of the molecule is less sterically hindered. Reaction 4 in Fig. 7b is particularly interesting in that it is a very specific, stereochemically oriented reaction in which the R<sub>1</sub> mesityl and R<sub>2</sub> mesityloxy groups are removed with subsequent ketone formation at R<sub>1</sub>.

Model compounds of the trichothecene “active” site

A study of the chemistry of the trichothecan ring system compounds would not be complete without an understanding of what makes the molecule “tick.” Evidence has been presented (vide supra) that portrays the R groups as contributing to trichothecene toxicity. The 9,10-olefinic portion of the toxin molecule also influences toxicity since reduction by hydrogenation leads to a reduction in activity (31, 43, 52). However, a total loss of activity is realized when the epoxide function is destroyed (7, 16, 26, 171). As a further means of addressing this subject, model compounds [1,5-dioxaspiro(2,5) octanes] containing the oxirane function were produced and evaluated as trichothecene mimics of anticancer (cytostatic) activity. Figure 8 portrays eight compounds modeling the active site of the family of trichothecenes. Two studies were undertaken in the assessment of activity, the first being the Ehrlich ascites carcinoma activity screen (45) in mice. Only structures 2 and 3 imparted significant activity (Fig. 8), while compounds 6 and 8 were slightly active. The rest displayed no activity. Compound 2, the most active model, was 25 times less active than T-2 toxin. These results suggest that sterically hindered epoxides presumably have high activity along with the specific epoxide stereochemistry as illustrated between 3 and 4. The four most active spirooctanes were then screened for lymphocytic leukemia P388 activity. They all displayed slight
Figure 7. Chemical reactions of the trichothecenes: (a) oxidation reactions.
Figure 7. Chemical reactions of the trichothecenes: (a) oxidation reactions.
Selective oxidation of the allylic alcohol occurs.

Other hydroxyl groups present must be protected by the acetyl groups.

Allylic methylene oxidation

The acetyl moieties act as protecting groups if the original trichothecene had a hydroxyl group.

Li:diisopropylamide or Li:hexamethyldisilazide

Trimethylsilyl trifluoromethanesulfonate

OsO₄/N-methylmorpholine N-oxide

The R₅ ester is more susceptible to oxidation by SeO₂ than the other positions containing an ester group.

Figure 7. Chemical reactions of the trichothecenes: (a) oxidation reactions.
Figure 7. Chemical reactions of trichothecenes: (b) reduction reactions.
Figure 7. Chemical reactions of trichothecenes: (c) Lewis acid and base reactions.
1. **ACETYLATION**

   APOVERRUCAROL

   \[ \text{Ac}_2\text{O} \quad \text{pyr} \quad \rightarrow \quad \text{AcO} \quad \text{OH} \quad \text{OAc} \]

2. **CATALYTIC HYDROGENATION**

   VERRUCAROL

   \[ \text{Pd} \quad \text{H}_2 \quad \rightarrow \quad \text{OH} \]

3. **HYDRATION, RING CLOSURE**

   VERRUCAROL

   \[ \text{H}_2\text{O} \quad 100^\circ\text{C} \quad 6\text{hr.} \quad \rightarrow \quad \text{CH}_2 \]

4. **RING CLOSURE**

   APOVERRUCAROL

   \[ \text{MnO}_2 \quad \rightarrow \quad \text{OH} \quad \text{O} \]

Figure 7. Chemical reactions of trichothecenes: (d) other reactions.
activity with compound 2 registering at a maximum (3,30).

Chemical reactions of trichothecene epoxide and epoxide-containing compounds

To the best of the author’s knowledge, no systematic study of the chemical reactions of the oxiran ring of the toxins has been reported, including such parameters as kinetics and temperature and solvent effects with various agents. The literature describes trichothecene-epoxide reactions dealing primarily with simple acids (e.g., HCl, H$_2$SO$_4$, HBr, and CF$_3$COOH) (43,50,53), bases (e.g., KOH, NaOH, Na$_2$CO$_3$, and NH$_4$OH) (50), and oxidation (43,68) and reduction (53) reactions. In particular, it has been observed that, using either concentrated HCl/EtOH and concentrated HBr/aq (43) or H$_2$CrO$_4$/acetone (53), trichothecin and verrucarol, respectively, entered into reactions rapidly (within minutes) at room temperature. However, these reactions exhibit a number of disadvantages including prolonged reaction times (slow rates of reaction), high temperatures and harsh reagents such as concentrated acids or bases.

Recently, a group of investigators (17) reasoned that since the 12,13-epoxide group is rather resistant to intermolecular attack, an intramolecular attack in addition to a concomitant 9,10-double bond rearrangement was sought for the epoxide-containing toxins. 2-Substituted thiazolium salts were chosen in which the carbon-4 hydroxyl moiety found on a number of trichothecenes would attack the 2-position on the thiazolium compounds. Expulsion of the leaving group (R) would then lead to intramolecular destruction of the oxiran ring. Essentially, thiazolium salts with the alkylthio R group were rather toxic (ip LD$_{50}$ of 70 mg/kg). An ip dose of 40 mg/kg (no deaths when administered alone) was given 15 min before injection of an LD$_{50}$ of anguidine (24 mg/kg). All mice given both compounds succumbed, leading to the conclusion that the thiazolium seemed to be the cause of the enhanced anguidine toxicity, therefore the function was changed to a substituted amine. The di-substituted amine (R group) derivative of the thiazolium salt displayed spectator status. No effect on anguidine activity was observed. Thus it was concluded that the thiazolium compounds augmented or had no effect on anguidine toxicity as opposed to neutralizing the molecule’s toxic properties.

Epoxide ring reactions of the trichothecenes have been discussed, while attention will now turn to potential reagent/reaction conditions suitable in the destruction of the trichothecene epoxide ring. Better conditions are deemed necessary because of the limitations imposed by present reaction schemes. It is possible to chemically target the ring functionality, which is important because it occupies a central role in toxicity. This ease of defining the oxiran ring functionality is in contrast to the various R groups and the olefinic bond which, as a whole, topically exist in a more diverse arrangement about the trichothecene molecule. Modifying either one or several of the groups reduces toxicity as opposed to eliminating it with the destruction of the epoxide ring.

Greater understanding of some of the reactions concerning their mechanistic behavior could be of significance. Under basic or neutral conditions, the mode of attack of a nucleophile follows an anti-coplanar arrangement of the reagent, oxygen, α-carbon and β-carbon atoms in the epoxy ring (Fig. 9). An S$_{N}2$ reaction takes place in that the nucleophile approaches the least substituted carbon (β - C). Under acidic conditions, protonation of the ring oxygen occurs, and the nucleophile attacks the α-carbon in order to relieve its electron debt, following an S$_{N}1$-type reaction.

Talmage et al. (160) reported a number of potential reagents that could allow the destruction of the epoxide ring under relatively mild reaction conditions. Some of these are the supernucleophile sodium thiophenoxide in DMF or DMSO, the nucleophiles sodium thiocyanate or hydroxylamine in aqueous systems, sodium borohydride, and the “super acids” trifluoromethanesulfonic acid (CF$_3$SO$_3$H) and “magic acid” (HBF$_4$ + SbF$_5$). However, among the myriad of epoxy-compound reactions surveyed by the author, several more appear to be of potential use in the convenient, rapid and mild destruction of the trichothecene epoxide ring (Table 9). Even though the
reagents vary somewhat, the reaction conditions are similar. Solvent systems range from polar to nonpolar, yet ambient temperature prevails and reaction completion times are short. Consequently, it is obvious that these reagents should be screened for trichothecene-epoxide destruction.

**BIOCHEMICAL METHODS OF ANALYSIS OF TRICHOTHECENES**

It was shown in an experiment that glutathione S-epoxide transferase catalyzes the conjugation reaction between a trichothecene and an excess of the thiol enzyme gonadotropic stimulating hormone (GSH) (42). The amount of toxin bound was measured indirectly by determining the remaining concentration of GSH by reacting it with 5,5-dithiobis(2-nitrobenzoic acid) and then measuring the rate of formation of the colored ion of 5-mercaptol(2-nitrobenzoic acid). Submicrogram quantities could be detected by this biological technique (157). In a similar vein, the trichothecene epoxide group also reacts with the thiol side group or the enzyme alcohol dehydrogenase (178). A reliable assay technique (174) was designed in which concentrations of 5 to 60 μg of T-2 toxin/ml could be determined by applying solutions of unknown concentrations of T-2 toxin and standard solutions of the toxin onto the shaved backs of test and control rats and noting the relative intensities of the reactions. Another method used is the rabbit reticulocyte assay. This technique involves the inhibition by the trichothecenes of 14C-leucine uptake by

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**TABLE 9. Potential Reagent-Reaction Conditions for the Trichothecenes**

<table>
<thead>
<tr>
<th>Epoxide compound</th>
<th>Reagent</th>
<th>Solvent</th>
<th>Reaction time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td><img src="image" alt="Epoxide Structure" /></td>
<td>HgSO4</td>
<td>H2SO4</td>
<td>Immediate</td>
<td>100</td>
</tr>
<tr>
<td><img src="image" alt="Epoxide Structure" /></td>
<td>LiNEt2</td>
<td>Ether-hexane</td>
<td>Immediate</td>
<td>168</td>
</tr>
<tr>
<td><img src="image" alt="Epoxide Structure" /></td>
<td>BF3·OEt2</td>
<td>Benzene</td>
<td>1 min</td>
<td>133</td>
</tr>
<tr>
<td><img src="image" alt="Epoxide Structure" /></td>
<td>BF3·OEt2</td>
<td>Benzene</td>
<td>20 min</td>
<td>65</td>
</tr>
<tr>
<td><img src="image" alt="Epoxide Structure" /></td>
<td>HClO4</td>
<td>1:9 Dioxane-H2O</td>
<td>–</td>
<td>145</td>
</tr>
<tr>
<td><img src="image" alt="Epoxide Structure" /></td>
<td>LiBEt3H</td>
<td>THF</td>
<td>5 min</td>
<td>15.85</td>
</tr>
</tbody>
</table>

* All reactions were performed at ambient temperature (20°-25°C).
the cells. Toxin concentrations as low as 30 ppb have been detected (172,179). The main drawback of these methods of trichothecene detection is that, although sensitive, they lack selectivity and specificity. However, this disadvantage has been overcome in the use of the enzyme-linked, immunosorbent assay technique (ELISA) (90,125,126). In a relatively simple procedure, the toxin of interest is conjugated to either bovine serum albumin or horseradish peroxidase. The antibody sera are applied onto polystyrene microtissue culture plates simultaneously with conjugated standards on other plates. Minimum detection levels of this technique are on the order of 2.5 pg/assay. This extremely sensitive and specific assay has a disadvantage in that preparation of specific antibodies to a particular toxin usually occurs over a period of several years.

The fate of administered toxins in animals was studied previously with radioactively labeled compounds. 3H-labeled fusarenon-X was observed to spread rapidly to the intestines, liver, and other organs of the body. Within one day, 25% of the radioactivity was eliminated in the urine and upon analysis yielded nivalenol (97,119,184). The same treatment with 3H-labeled T-2 toxin produced HT-2 toxin with no detectable amount of T-2 toxin. Upon subsequent analysis, both radioactive compounds were found to be deacetylated at the R2 function in the liver by microsomal esterase (119). Nakano et al. (110) conducted experiments consisting of iv injections, into mice, of the basic trichothecene compound (R1 = R2 = R3 = R4 = R5 = H) which was 14C-labeled on the epoxide ring. The observed high radioactivity in the liver and kidney decreased rapidly, although it persisted in the bladder and intestines. Per os (oral) administration to the mother led to its appearance in the 7-d-old suckling mice stomachs, thus showing secretion of the toxin in the milk. Also, in rats, no radioactive 14CO2 was detected in the expired breath when given either orally or intravenously, indicating no cleavage of the epoxide ring. Another study (88) showed that labeled T-2 toxin injected into pregnant rats produced radioactivity in the thymus of the fetus and persisted for 1 week.

The effect of T-2 toxin action on DNA was studied by using Parodi’s alkaline elution technique coupled with a microfluorimetric DNA determination (89,122,157). Briefly, the experiment relies on the fact that the greater the incidence of toxin-induced DNA cleavage, the greater the number of smaller DNA fragments are produced which subsequently elute through cellulose filters. The toxin was utilized in vivo and in vitro, and its effects were studied on liver, spleen, and thymus tissues from rats. In both instances, no damage was observed on the hepatic DNA. However, with concentrations as low as 5 ng/ml of culture and 2-h exposure times, the lymphoid organs were extensively damaged in that many breaks in the cellular DNA occurred. In vivo, as opposed to in vitro, reversibility occurred which indicated that DNA repair took place. Because other chemical compounds (157) such as methylnitrosourea and methyl methanesulfonate require concentrations of approximately 0.1 mM to induce DNA breaks, T-2 toxin is indeed a very potent substance.

Structure-activity relationships from in vitro studies

The trichothecenes are the most potent protein synthesis inhibitors known for eucaryotic cells. They inhibit either the initiation or termination step of protein synthesis on the cellular level. Table 10 summarizes at what stage different trichothecenes exert their inhibitory effect. The mode of action the trichothecenes undertake at the protein synthesis level can be explained by two biochemical models, one being favored over the other (123,192). A synopsis of the preferred model shows that the toxin binds throughout the protein synthesis cycle with equal affinities, only interfering with the crucial enzyme, peptidyl transferase, when the ribosome unit assumes a certain three-dimensional structure. Two of the substituent side groups of the toxin would determine the site of activity on the ribosomal unit (20,191). Analyzing these facts (146,192), structural correlations can be assumed. R2 substitution (R1 = R3 = H) is detrimental (inhibits peptidyl transferase activity) at the elongation or termination stage. R1 and R3 residing on the opposite side of the R2 function in the toxin molecule (cf. Fig. 1), inhibit initiation when derivatized (OH, OAc). The toxin verrucarol (R1 = H, R2 = R3 = OH) prevents elongation while inhibiting initiation of protein synthesis when esterified. The vital structure-activity relationships of this model are: (a) R2 substitution inhibits elongation and termination and (b) R1 and R3 substitution leads to initiation inhibition. Since the epoxide ring, which is crucial for toxicity, is also found on the same side of the molecule as the R1, R2, and R3 functions, the toxin’s ability to impart deleterious effects appears to be located on the right half of the molecule (Fig. 1).

**Table 10. Trichothecenes and Their Stage of Protein Synthesis Inhibition**

<table>
<thead>
<tr>
<th>Initiation</th>
<th>Elongation or termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarenon-X</td>
<td>Verrucarol</td>
</tr>
<tr>
<td>Nivalenol</td>
<td>Crotocin</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>Crotocin</td>
</tr>
<tr>
<td>Scirpentriol</td>
<td>Trichothecolone</td>
</tr>
<tr>
<td>MAS</td>
<td>Trichoderm</td>
</tr>
<tr>
<td>DAS</td>
<td>Trichoderm</td>
</tr>
<tr>
<td>Verrucarin A.E,J,H</td>
<td>Trichodermol</td>
</tr>
</tbody>
</table>

**SUMMARY**

The historical and pathological aspects of the trichothecene mycotoxins document and portray, respectively, the need for an increased awareness and understanding of these ubiquitous toxins. They continue to pre-
sent a challenge to investigators in many scientific fields including analytical and organic chemistry, biochemistry and toxicology. Various areas in these fields were discussed in terms of their established, current and technological methodologies. For example, state-of-the-art in analytical instrumentation and derivatization techniques have the potential of increasing the selectivity and sensitivity of trichothecene detection. Other avenues in the realm of organic chemistry exist which could provide powerful yet relatively mild reaction conditions to effect the elimination of trichothecene toxicity, and present biochemical work has provided an increased understanding in the link between the structure of the toxins and the cellular sites that are sensitive to toxin presence.

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