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MODE OF ACTION OF TRICHOTHECENES

Yoshio Ueno

Department of Microbial Chemistry, Faculty of Pharmaceutical Sciences,
Tokyo University of Science, Ichigaya, Tokyo 162, Japan

Abstract - In the present discussion, the author summarized the toxicological and biological features of thirty kinds of trichothecene mycotoxins which are produced by a wide range of Fusarium, Myrothecium and others. The 12, 13-epoxytrichothecenes induce nausea, vomiting, skin inflammation, leukopenia, diarrhea, haemorrhage in lung and brain, and destruction of bone marrow. Since these toxicological characteristics coincide with a major symptom of intoxicated humans and farm animals induced by consumption of moldy cereals and feeds, the red-mold toxicosis and bean-hulls poisoning in Japan, moldy corn toxicosis in USA, alimentary toxic aleukia (ATA), stachybotryotoxicosis and dendrodochiotoxicosis in Europe, are originated from a common toxicant, trichothecenes. Orally administered trichothecenes are rapidly absorped and eliminated into the feces and urine upon deacylation at C-4 and C-8 by the microsomal esterase and others. Biochemical approaches to the mode of action revealed that the trichothecenes are a potent inhibitor of protein and DNA syntheses in eukaryotic cells. Binding to the eukaryotic polysomes and ribosomes and the subsequent inactivation of ribosomal cycle is responsible for their inhibitory effect to initiation and termination reactions. Microbial approaches revealed that the trichothecenes are mutagenic to yeast cells, but are negative in DNA-attacking ability to Bacillus subtilis and reversion assay with Salmonella typhimurium. Reactivity of the epoxide ring of trichothecenes with SH-group of proteins will be discussed in relation to the molecular mechanism of action.

INTRODUCTION

The trichothecenes are a chemically related group of fungal metabolites produced by various species of Fusarium, Myrothecium, Trichoderma, Cephalosporium, Verticimonosporium and Stachybotrys. These fungi invade various agricultural products, and upon consumption of molded cereals as food and feeds, a serious intoxication of humans and farm animals develops. In view of the coincidence of major symptoms in naturally-occurring intoxication and experimentally-induced toxicological features, the author became convinced that the trichothecene compounds are a common toxicant in Akakabi (red-mold)-toxicosis and bean-hulls poisoning in Japan, alimentary toxic aleukia in USSR, moldy corn toxicosis in USA, and stachybotryotoxicosis in Central Europe. Dendrodochiotoxicosis of horses in Europe is also presumed to be induced by trichothecenes, since the responsible fungus, Dendrodochium toxicum is synonymous with Myrothecium roridum which is able to produce a macrocyclic trichothecene. All the trichothecene compounds are potent inhibitors of protein and DNA synthesis in eukaryotic cells, and this biochemical characteristic is responsible for their potent cytotoxicity. Inactivation of initiation and termination reactions of protein synthesis is caused by the binding of trichothecene to ribosomal structure elements. Reactivity of trichothecenes with the SH-group of proteins will be discussed with relation to their toxicity.

RESULTS AND DISCUSSIONS

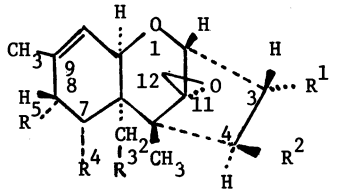
Toxicological importance of trichothecene mycotoxins: Trichothecene compounds are produced by many species of fungi such as Fusarium, Myrothecium, Stachybotrys and others. The first trichothecenes, verrucarins, were isolated during a search for antibiotics in 1946, and diacetoxyscirpenol represents the earliest isolation of phytotoxic trichothecene. Recent toxicological surveys have demonstrated a wide range of trichothecene metabolites from the above fungal flora. Historically, a noteworthy report was published by Woronin in 1891 (Ref. 1). He described in detail the "Taumelgetreide (staggering grains)" intoxication that affects humans and domestic animals, and implicated Fusarium roseum, Gibberella saubinetti, Helminthosporium spp. and Cladosporium herbarum as causal fungi. Similar poisonings due to moldy cereals and grains also developed in the USSR, Central Europe, Finland, North America, China and other districts where barley, millet, wheat or corn was consumed as a major foodstuff. In 1942-1947, over 10 % of the population of Orenburg (west of Siberia) were fatally

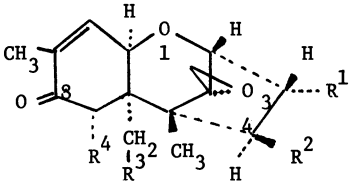
affected by overwintered millet and other kinds of cereals. Since the number of white blood cells of the patients decreased markedly, the diagnostic name "Alimentary Toxic Aleukia (ATA)" was given to this disease. As for the causal fungi and toxicants, F.sporotrichioides and F.poae, and steroidal and thiol derivatives were reported (Ref. 2). The present author has demonstrated that these fungi possess the ability to produce trichothecene mycotoxins such as T-2 toxin and neosolaniol (Ref. 3). The presence of T-2 toxin in the sample "Poaefusarin" was chemically proved (Ref. 4). In USA, several investigators reported a heavy intoxication by moldy cereals, and from the toxic fraction of cultured F.tricinctum T-2 toxin was isolated (Ref. 5). In Japan, "red-mold disease" or "wheat scab" has long been known as a disease of wheat, barley, oats and rye, and upon ingestion of these cereals as food or feed, serious diseases characterized by vomiting, diarrhea, refusal of feed, congestion or haemorrhage in the lung, adrenals and intestines, were developed (Ref. 6), and from the metabolites of F.nivale, one of the causal fungi of "Akakabi disease" of barley, nivalenol and fusarenon-X were isolated (Ref. 7 & 8). Recent researches on stachybotryotoxicosis, which is known as a severe disease of horses and other kinds of farm animals in Europe, demonstrated the several macrocyclic trichothecenes such as satratoxins (Ref. 9). Similar poisonings of horses have developed in Hokkaido, a northern island of Japan, where a massive breeding of horses was carried out during the second World War, and on ingestion of molded bean-hulls, fetal cases as well as nervous disorderes such as convulsion, cyclic movements and retarded reflexes developed. Microbial and chemical analysis demonstrated T-2 toxin and other kinds of trichothecenes from F.solani etc. (Ref. 10). From these observations, the author pointed out the toxicological importance of trichothecene compounds in the development of various intoxications in the World, as summarized in Table 1.

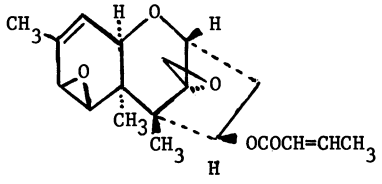
TABLE 1. Major symptoms and suspected fungi in trichothecene toxicosis in the world

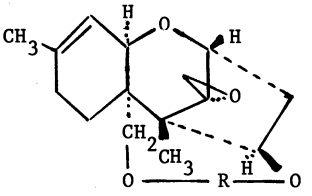
Toxicosis	District and affected species	Fungus	Symptoms
"Taumelgetreide" toxicosis	Siberia man, horse, pig, fowl,	G.saubinetti	headaches, chills, nausea, vomiting,
Red-mold toxicosis	Japan man, horse, pig, cow,	F.graminearum	vomiting, haemorrhage, refusal of feed,
Moldy corn toxicosis	USA pig, cow,	F.tricinctum	emesis, vomiting, refusal of feed,
Alimentary Toxic Aleukia	USSA man, farm animals,	F.sporotrichioides	vomiting, inflammation, leukopenia, diarrhea, necrotic angina,
Stachybotryotoxicosis	USSR horse,	Stachybotrys atra	shock, somatitis, dermal necrosis, leukopenia,
Bean-hull toxicosis	Japan horse	F.solani etc.	convulsion, cyclic movement,
Dendrodochiotoxicosis	USSR sheep, pig,	Dendrodochium toxicum	inflammation, haemorrhage,

Chemical characteristics of trichothecenes : The trichothecene compounds comprise a group of closely related sesquiterpenoids and contain trichothecane nuclei. The naturally occurring compounds possess an olefinic bond at C-9 and 10, and an epoxy ring at C-12, 13. Thus, they are characterized as 12,13-epoxytrichothecenes. Trichodermol, the simplest structure in the family has a hydroxy group at 4, while the remaining members are formed by further hydroxyl, acyloxy, epoxy, carbonyl or macrocyclic substitution at 3, 7, 8 and 15. At the present time, around thirty kinds of derivatives have been isolated. The author classified the toxins into four goups; the type A (T-2 toxin et al.), the type B (nivalenol et al.), the type C (crotocin) and the type D (macrocyclic compounds) (Fig. 1) (Ref. 11). On alkali treatment, the esters are saponified and the resulting alcohols lead to decrease in biological and toxicological activity. The 12,13-epoxide ring is cleaved on strong mineral acid treatment, resulting in loss of activity. The hydrogenation of the 9-10 double bond induces a slight decrease of activity. In general, the type A and D toxins are higher in cytotoxicity than the type B and C toxins, indicating the importance of C-8 substitution and macrocyclic ring.

(A) 

(B) 

(C) 

(D) 

		R^1	R^2	R^3	R^4	R^5
(A)	Trichothecene	H	H	H	H	H
	Trichodermol (roridin C)	H	OH	H	H	H
	Trichodermin	H	OAc	H	H	H
	Dihydrotrichothecene	H	OH	H	H	OH
	Verrucarol	H	OH	OH	H	H
	Scirpentriol	OH	OH	OH	H	H
	T-2 tetraol	OH	OH	OH	H	OH
	Monoacetoxyscirpenol	OH	OH	OAc	H	H
	Diacetoxyscirpenol	OH	OAc	OAc	H	H
	8α-Hydroxy-diacetoxyscirpenol (Neosolaniol)	OH	OAc	OAc	H	OH
	Monoacetylneosolaniol	OH	OAc	OAc	H	OAc
	7α-Hydroxydiacetoxyscirpenol	OH	OAc	OAc	OH	H
	7,8α-Dihydroxydiacetoxyscirpenol	OH	OAc	OAc	OH	OH
	T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
	HT-2 toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	Acetyl T-2 toxin	OAc	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
(B)	Nivalenol	OH	OH	OH	OH	
	Monoacetylnivalenol(fusarenon-X)	OH	OAc	OH	OH	
	Diacetylnivalenol	OH	OAc	OAc	OH	
	Deoxynivalenol	OH	H	OH	OH	
	Monoacetyldeoxynivalenol	OAc	H	OH	OH	
	Trichothecin	H	$OCOCH=CHCH_3$	H	H	
(C)	Crotocin		$OCOCH=CHCH_3$			

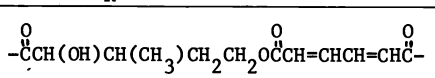
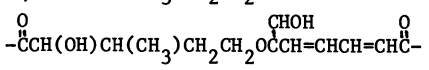
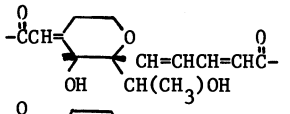
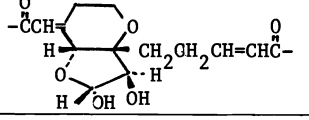
		R
(D)	Verrucarin A	
	Roridin A	
	Satratoxin H	
	Vertisporin	

Fig. 1. Naturally-occurring trichothecenes

Toxicological characteristics of trichothecenes: The fusariotoxicosis described above are characterized by common symptoms such as emesis, vomiting, inflammation, haemorrhage, refusal of foodstuffs, diarrhea, abortion, haematological changes, necrotic angina, nervous disorder, and destruction of bone marrow. Experimental approaches to the trichothecene compounds have revealed that these symptoms are induced in laboratory animals such as mice, rats, guinea-pigs, rabbits, cats, dogs and avians, although the toxicological features vary according to the animals and mycotoxins used (Ref. 12). In general, the acute toxicity to young or immature animals is higher than to adults. No marked sex difference is observed. Nervous disorders such as emesis, vomiting, and refusal of feed are induced in ducklings (Ref. 13) and pigs (Ref. 14). Refusal of feed is one of the characteristic symptoms of fusariotoxicosis, and T-2 toxin is reported to induce feed refusal in rats (Ref. 15). However, the author demonstrated that T-2 toxin and macrocyclic trichothecenes are highly active in their inflammatory effect, and the resulting injury to the oral cavity and mucous membranes of the stomach and intestines results in decrease of feed consumption as well as body weight. Subchronic ingestion of trichothecenes causes a decrease of circulating white blood cells in cats (Ref. 16). This leukopenic change of animals is reported as a characteristic symptom of ATA disease.

All the trichothecene compounds are proved to induce skin necrotization (Ref. 17, 18). Oral lesions in poultry (Ref. 19) and papilloma formation in the stomach of rats (Ref. 20) are presumed to be caused by the mucous irritability of trichothecenes.

The structure-toxicity relationships in the trichothecenes were summarized in Table 2. The acute toxicity to animals, toxicological effects and cytotoxicity are the highest in the macrocyclic trichothecenes (D) followed by the (A) toxins, the (B) toxins and the (C) toxins. Tests with cultured cells revealed the hypothesis that cytotoxicity is associated with the 12, 13-epoxytrichothecene nucleus, but other structural features, notably the presence of a 9-ene and esterification of some, though not all, of the hydroxyl substituents are important factors in the manifestation of high toxicity. In the case of nivalenol, the stepwise acetylation increases the toxicity to chick embryo as well as the cytotoxicity. Although it is tempting to attribute this result to the influence of increasing lipoidal solubility as the number of acetate residues is increased, this is unlikely to be the complete, or indeed the correct, explanation. For example, the fully acetylated tetraacetylnivalenol exhibits less toxicity. Therefore, it is necessary that the biotransformation of trichothecenes should be clarified.

TABLE 2. Comparison of the toxicological and biological activities of trichothecenes

(a) Toxicity

Trichothecenes	LD_{50}		Vomiting		Skin inflammation
	mice (mg/kg i.p.)	chick embryo (μg/egg)	ducklings (mg/kg s.c.)	cats	guinea-pigs (μg)
(A) T-2 toxin	5.2	0.07	0.1	0.1	0.2
HT-2 toxin	9.0	0.5	0.1		0.2
Diacetoxyscirpenol	23	0.09	0.2		0.2
Neosolaniol	14.5	5.0	0.1		1
(B) Nivalenol	4.1	4.0	1.0		10
Fusarenon-X	3.3	2.6	0.4	1	1
Diacetylnivalenol	9.6	1.9	0.4		1
Deoxynivalenol	70		13.5		
(C) Crotocin	700 (i.v.)				10
(D) Verrucarin A	1.5 (i.v.)				0.05
Roridin A	1.0 (i.v.)				0.05
			(Ref.13)	(Ref.16)	(Ref.17)

(b) Cytotoxicity

Trichothecenes	ID_{50} (μg/ml)	MED (μg/ml)	
	Tetrahymena pyriformis	HeLa	HE_p2 cells
(A) T-2 toxin	0.05	0.01	0.001
HT-2 toxin	0.5		
Diacetoxyscirpenol	0.05		
Neosolaniol	0.5		
(B) Nivalenol	10	0.2	0.2
Fusarenon-X	5	0.1	
Diacetylnivalenol	1	0.1	
Deoxynivalenol	5		
(D) Verrucarin A			0.001
Vertisporin		0.001	
	(Ref.21)	(Ref.22)	

Fate and metabolism of trichothecenes : From the toxicological evidence that the LD_{50} values (mg/kg) of fusarenon-X to mice are very similar irrespective of different administration routes (i.v. 3.4; s.c. 4.2; i.p. 3.4; p.o. 4.5), the author presumed that the trichothecene compounds are very effectively absorbed to reach the target organ(s). Experiments with labelled fusarenon-X demonstrated that the toxin is quickly absorbed from the intestine and excreted into faeces and urine (Ref. 12). A similar result was obtained with T-2 toxin. Chemical analysis of the faeces and urine have demonstrated that fusarenon-X and T-2 toxin are excreted as the deacylated derivatives, nivalenol, HT-2 toxin and neosolaniol(Ref. 23). Further experiments with the subcellular fractions of livers from rats and others indicated that fusarenon-X and T-2 toxin are deacetylated at C-4 by the microsomal enzyme (Fig. 2) (Ref. 24). Therefore, it is highly possible that the trichothecenes such as fusarenon-X and T-2 toxin are biotransformed by the microsomal enzyme of liver. Since the deacetylation is inhibited by eserine and diisopropylfluorophosphate, acetylcholine esterase-type enzyme catalyses this conversion. Comparative survey has demonstrated that the esterase activity to trichothecenes is the highest in the microsome of rabbit, followed by human and rat (Table 3).

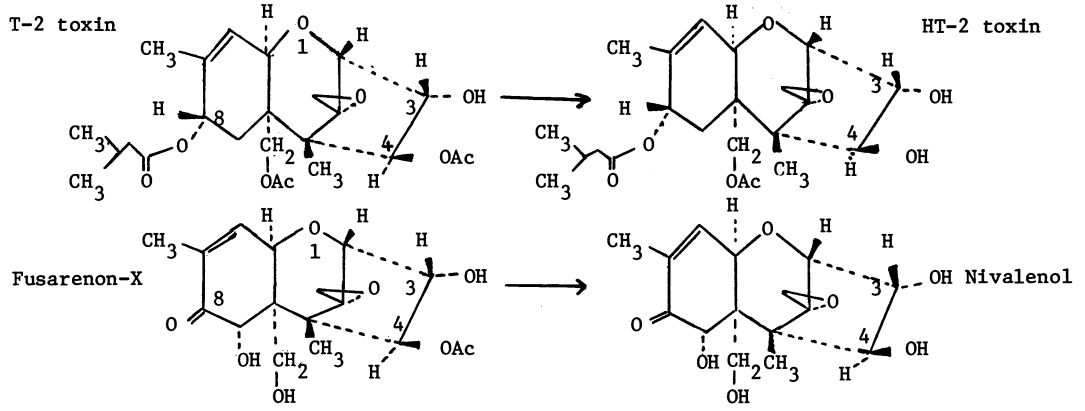


Fig. 2. Microsomal deacetylation of T-2 toxin and fusarenon-X

TABLE 3. Species and organ differences in the microsomal deacetylation of T-2 toxin

	HT-2 toxin formed (μ mole/mg protein/10 min)		
	human	rat	rabbit
Liver	331	31	3 401
Kidneys		1	1 571
Spleen		1	73
Brain		10	25
Serum		0	

Biochemical mode of action : Since the trichothecene mycotoxins induce a cellular damage characterized by karyorrhexis and destruction of the actively dividing cells in thymus, testis, intestines, spleen and others, the author believed that this type of mycotoxins interferes with the synthesis of macromolecules. Actually, during the survey of toxic principles of F. nivale-molded rice, the author showed that the crude extract inhibited the protein synthesis in rabbit reticulocytes and the following analysis with this bioassay revealed nivalenol as a toxic agent (Ref. 25). This report is the first approach to the biochemical features of trichothecenes. Comparative toxicology with various kinds of trichothecenes has revealed that all the compounds inhibit the protein and DNA synthesis in reticulocytes (Ref. 26), Ehrlich ascites tumor (Ref. 27), cultured cells (Ref. 28), and protozoa (Ref. 21). No inhibitory effect on bacterial multiplication is correlated with the fact that the toxin exhibits no inhibition of synthetic reaction in bacterial cells.
A tracer experiment with the whole cells of reticulocytes revealed that the 50 % inhibition concentration for protein synthesis varies widely according to the chemical structure. The most effective compounds are verrucarin A and roridin A, which cause ID_{50} at a low concentration of 10^{-8}M (Ref. 29) (Table 4), and followed by the (A) toxins, the (B) toxins and the (C) toxin. In vitro experiments with the isolated polysomes and ribosomes of reticulocytes and liver, however, revealed no marked difference in the inhibitory concentration (Ref. 30). This evidence is explained by the fact that lipophilic T-2 toxin has higher affinity to the cellular membranes than the hydrophilic fusarenon-X (Ref. 31).
An early effect of the trichothecenes on animal cells is to disaggregate the polysomal structure (Ref. 31). This phenomenon is inducible by a short-term contact of the cell with

the toxin and is not reversed by the further incubation of the cells in the absence of toxin. The experiments with labelled T-2 toxin and fusarenon-X revealed that the toxins bind to polysomes and ribosomes from eukaryotic cells. Since the eukaryotic cells possess 80 S ribosomes, the affinity of trichothecenes to animal cells is attributable for their high affinity to 80 S ribosomes (Ref. 31). The binding of trichothecenes depends on the structural condition of ribosomes; the highest binding was observed with microsomes, followed by active polysomes and ribosomes. No detectable binding was observed with the subunits of ribosomes (Table 5) (Ref. 32).

TABLE 4. Inhibition of protein synthesis in rabbit reticulocytes and rat liver

	Trichothecenes	ID_{50} (μg/ml)		
		reticulocytes		rat liver
		whole cells	cell-free	cell-free
(A)	T-2 toxin	0.03	0.15	ca. 5
	HT-2 toxin	0.03		
	Diacetoxyscirpenol(DS)	0.03	5	50
	Neosolaniol	0.25	5	20
	7-hydroxy-DS	0.4		
	7,8-dihydroxy-DS	0.6		
(B)	Nivalenol	3.0	0.5	8
	Fusarenon-X	0.25	0.2	8
	Diacetylnivalenol	0.10		
	Tetraacetylnivalenol	10		
	Dihydronivalenol	3.0		
	Trichothecin	0.15		
	Trichothecolon	20		
(C)	Crotocin	0.45		
(D)	Verrucarin A	0.01		
	Roridin A	0.01		

TABLE 5. Binding of fusarenon-X to microsomal particles from rat liver (Ref. 32)

Particles	^{3}H-Fusarenon-X bound ($\mu g/A_{260}$)
Microsomes	0.53
Polysomes	0.33
Ribosomes	0.31
60 S subunit	0.02
40 S subunut	0.05

The accumulated evidence that the trichthecenes inhibit peptidyl polymerization and puromycin reaction, cause disaggregation of polysomes, and bind with active polysomes, leads to the conclusion that the mechanism of action is as follows;the trichothecenes interfere with the active center of peptidyl transferase of ribosomes and prevent the ribosomal cycle by causing the inhibition of initiation and termination reactions. Independent experiments also demonstrated the binding of trichodermin to polysomes and subsequent inhibition of peptide synthesis (Ref. 33, 34).

As for DNA synthesis, the author has demonstrated that the trichothecenes inhibit DNA synthesis in Ehrlich ascited tumor cells (Ref. 27), and a protozoa Tetrahymena pyriformis (Ref. 21). The synthesis in HeLa cells is also inhibited by the toxins (Ref. 35). As for the mechanism of inhibitory effect, no detailed information is available. At the present time, the author has proved that the toxins cause no inhibition of thymidine kinase and DNA-polymerase from the tumor cells. Elevation of TDP and TTP levels and an accumulation of short-piece DNA in the treated cells suggests an interference of ligase reaction in vivo (unpublished). The mycotoxins such as T-2 toxin, nivalenol and fusarenon-X show no inhibitory effect to RNA synthesis in ascites tumor cells (Ref. 27), cultured cells (Ref. 35) and protozoa (Ref. 21). Recent approaches in our laboratory also indicate that the trichothecenes inhibit neither nuclear RNA synthesis nor DNA-dependent RNA polymerase of rat liver. Noticeable findings are an interference with lipid synthesis and phosphate uptake in Tetrahymena pyriformis, which may suggest a possible impairment of membrane function by the trichothecenes (Ref. 36).

Reactivity of trichothecenes with SH-compounds : Epoxide groups of many biologically active compounds are able to react enzymatically or non-enzymatically with SH-groups of amino acids

and proteins. On this line, the author investigated the reactivity of trichothecenes with SH-enzymes. Selected enzymes were creatine phosphokinase, alcohol dehydrogenase and lactate dehydrogenase, all of which possess many thiol residues in the active center. On incubation of these enzymes in the presence of added substrates, no inhibition was obtained by the trichothecenes such as T-2 toxin, neosolaniol and fusarenon-X. However, marked inactivation occurs when the enzyme molecules are preincubated with the toxins followed by addition of the respective substrates. This inhibitory effect was prevented by prior addition of GSH or dithiothreitol. Gel-filtration of the mixture of enzyme protein and labelled toxin resulted in the complex formation with a molar ratio of 4 trichothecenes to one enzyme protein. Since alcohol dehydrogenase from yeast is proved to possess totally 36 SH-residues among which four residues are reactive with SH-reagent, from these considerations, the author considered that the trichothecene mycotoxins are able to react with the reactive SH-residue of enzyme protein (Ref. 37).
Recent progress on the molecular mechanism of drug metabolism indicates that an epoxide ring is inserted into a C-C bouble bond of aromatic compounds by microsomal oxygenase or epoxidase in the presence of NADPH and molecular oxygen, and the resulting epoxide is hydrolyzed by microsomal epoxide hydrolase, as presented in Fig. 3.

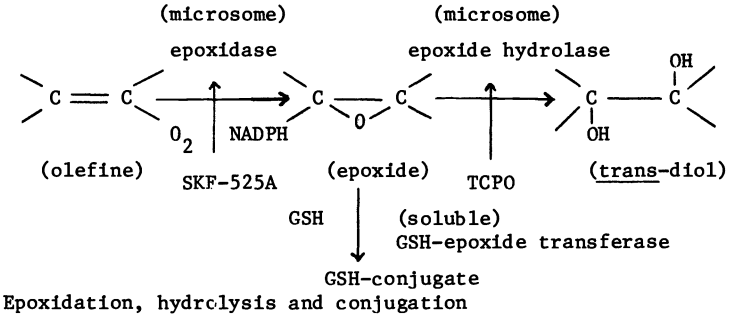


Fig. 3. Epoxidation, hydrolysis and conjugation

As well established in the case of aflatoxin B_1, this hepatocarcinogen is enzymatically activated by the oxygenase into 2,3-oxide and this active intermediate is presumed to bind to DNA and other macromolecules. From the fact that SKF-525A, a potent inhibitor of oxygenase, inhibits the activation and mutagenicity of aflatoxin B_1 and 1,2,3-trichloropropane oxide(TCPO) accelerates the binding of aflatoxin B_1 to DNA, the activities of epoxidase and epoxide hydrolase may control the level of active "epoxide". In regard to the effect of trichothecenes, the author investigated first the effect of trichothecenes on epoxidation reaction of aflatoxin B_1 and epoxide hydrolysis of safrole oxide. The trichothecenes such as fusarenon-X and T-2 toxin caused neither suppresion of aflatoxin B_1 mutagenicity to Salmonella typhimurium nor inhibition of safrole oxide hydrolysis. These findings indicate that the trichothecene compounds exhibit no affinity to epoxidase as well as epoxide hydrolase Another enzymatic reaction of an epoxide ring is mediated by glutathione transferase which is locallized in the soluble fraction of liver (Ref. 38). GSH-transferases catalyze the conjugation of GSH with a number of compounds bearing a nucleophilic group. At the present time, three enzymes have been purified homogenously; each is active with p-nitrophenethylchloride. One is active with epoxides and p-nitrophenethyl bromide; the second with methyl bromide; and the third with 3,4-dichloronitrobenzene and 4-nitropyridine N-oxide (Ref. 39). The author has investigated the GSH-transferase activity with the soluble fraction of liver and 1,2-epoxy-(p-nitrophenoxy)propane as substrate. Under the experimental conditions, the trichothecenes such as T-2 toxin and fusarenon-X caused no marked inhibition, indicating that GSH-transferase does not conjugate the trichothecenes with GSH. In this connection, by measuring the residual GSH level in reaction system of GSH-transferase, the reactivity of several trichothecenes such as T-2 toxin and diacetoxyscirpenol was presented (Ref. 40). In general, epoxide hydrolase and GSH-transferase exhibit substrate specificity, and the high affinity is demonstrable with the epoxide ring which is located in aliphatic side chains of substrates. In the case of trichothecenes, however, the epoxide ring is attached to an aromatic ring, and this chemical situation may influence their affinity to these enzyme molecules.

Mutagenicity of trichothecenes: The trichothecene compounds are one of the most important mycotoxins, and their carcinogenicity is of great concern in the present time. Long-term feeding experiments with mice and rats and a long-term exposure to skin tissues resulted in negative data in regard to carcinogenicity. Methodological progress on the rapid screening of carcinogenicity test is made by an introdution of bacterial mutants in combination with the activating enzyme system of liver. In this connection, the author has examined the DNA-attacking ability and mutagenicity of trichothecenes. A preliminary screening with mutant cells of Bacillus subtilis which lack recombination ability demonstrated that six Penicillium toxins (citrinin, penicillic acid, patulin, luteoskyrin, rugulosin and PR-toxin), five Aspergillus toxins (aflatoxin B_1 and G_1, sterigmatocystin, O-acetylsterigmatocystin and O-acetyldihydrosterigmatocystin) and two Fusarium toxins (zearalenone and zearalenol-b) were positive in the DNA-attacking ability. While, T-2 toxin and fusarenon-X are negative in this

Rec-assay (Ref. 41). Further experiments with Salmonella typhimurium TA 98 demonstrated the mutagenicity of aflatoxin B_1, G_1, sterigmatocystin and O-acetylsterigmatocystin in the presence of fortified S-9 fraction of the liver from rats which were induced by PCB. In the standard assay conditions, the trichothecenes such as T-2 toxin and fusarenon-X are negative. Noticeable finding is that crotocin is positive to TA 100. As shown in Fig. 1, crotocin (C) possess an additional epoxide at C-7, 8, which may play an important role for the mutagenicity (Table 6). A positive result was obtained by using yeast cells. On exposing the cells to nivalenol and fusarenon-X, respiratory-deficient (RD)-mutants or "petite" mutants were induced (Ref. 12). Another approach was made by using cultured cells. In this cell line, patulin and penicillic acid induce DNA-breakage, while the trichothecenes were negative (Ref. 43). Therefore, the mutagenicity of trichothecenes remain to be investigated in detail.

TABLE 6. Mutagenicity of mycotoxins to Salmonella typhimurium TA 98 (Ref. 42)

Mycotoxins	S-9*	Mutagenicity (μg/plate)						
		0	0.01	0.1	1.0	10	100	500
Aflatoxin B_1	+	-	+	+	+	-	-	
	-	-	-	-	-	+	+	
Aflatoxin G_1	+	-			+	+	-	
	-	-			-	-	-	
Sterigmatocystin	+	-			+	+	-	
	-	-			-	-	+	
O-Acetylsterigmato-cystin	+	-	-	+	+	-	-	
	-	-	-	-	-	-	-	
T-2 toxin	+	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Fusarenon-X	+	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Crotocin**	+	-			-	-	+	+
	-	-			-	-	-	-

* 9 000 g supernatant fraction of PCB-induced rat liver
** TA 100

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