CURRENT AWARENESS DOCUMENT

POLYNUCLEAR LACTONE-TYPE AND RELATED ALKYLATING AGENTS

CARCINOGENICITY AND STRUCTURE ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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5.3.1.2 Polynuclear Lactone-type and Related Alkylating Agents: Penicillium Toxins.

5.3.1.2.1 Introduction.

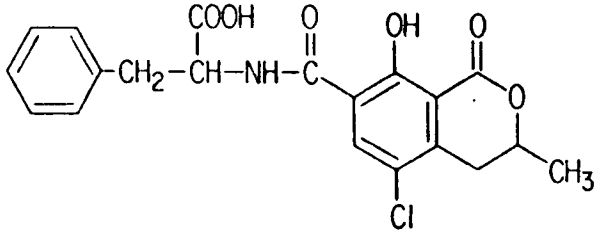
The Penicillium represent another important fungal group containing species which elaborate toxigenic as well as carcinogenic metabolites. Several Penicillium toxins, which have been tested for carcinogenic activity, are shown in Table XII.

Like the Aspergillus, members of the Penicillium group occur frequently as natural contaminants of foods and feeds and have been implicated as the causative agents in many instances of illness and death of humans and farm animals. A case in point is the outbreak of the "yellowed rice disease" in Japan shortly after World War II. The incident, which led to many deaths, has been attributed to rice heavily contaminated with P. islandicum, the mold that produces luteoskyrin, cyclochlorotine and islanditoxin. P. viridicatum, which elaborates ochratoxin A, citrinin, griseofulvin and penicillic acid, is one of the major contaminants of stored corn and various types of decaying vegetation (see rev. 1).

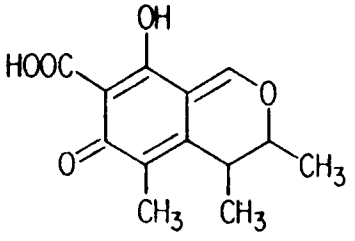
Early interest in the studies of griseofulvin, citrinin, patulin, penicillic acid, penicillin G and rugulosin arose largely because of their potential usefullness as antibiotics. Since the discovery of aflatoxin in the 1960's, awareness of the importance of natural chemicals as environmental contaminants has intensified; the biochemical, toxicological and human health effects of these and other mycotoxins have attracted dramatically increased attention in the last two decades. Several publications summarize current knowledge of these effects of Penicillium toxins (2-8).

Table XII

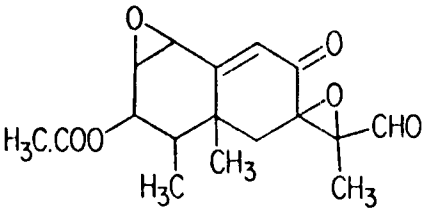
Penicillium Toxins Which Have Been Tested for Carcinogenic Activity



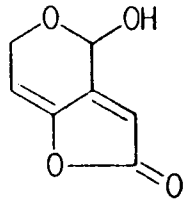
Ochratoxin A



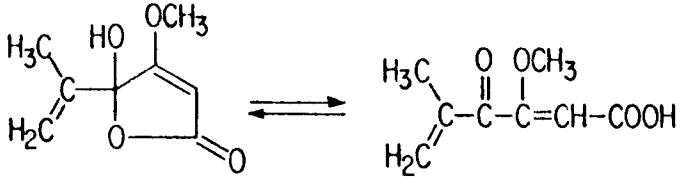
Citrinin



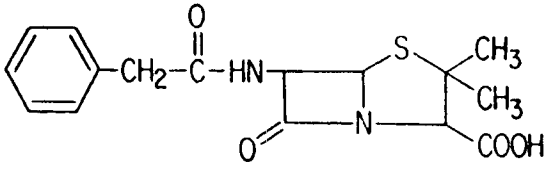
PR toxin



Patulin

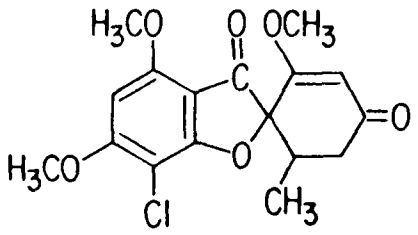


Penicillic acid

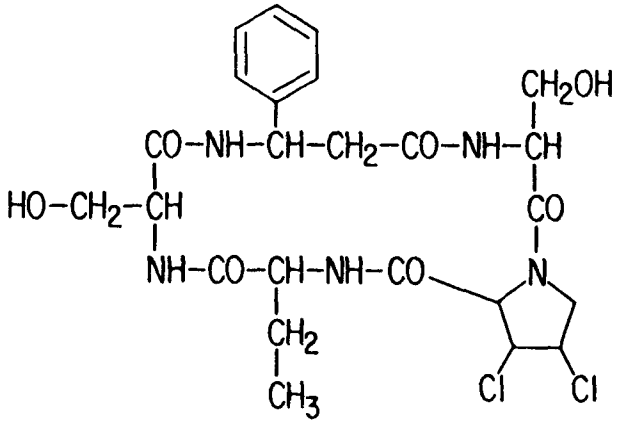


Penicillin G

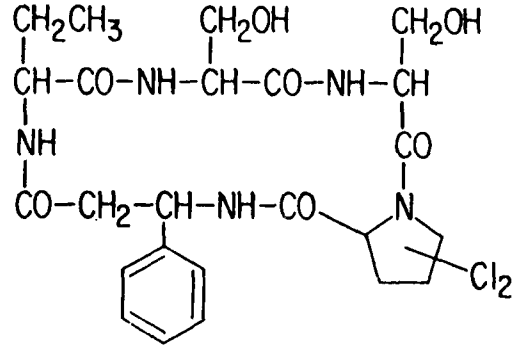
Table XII (Continued)



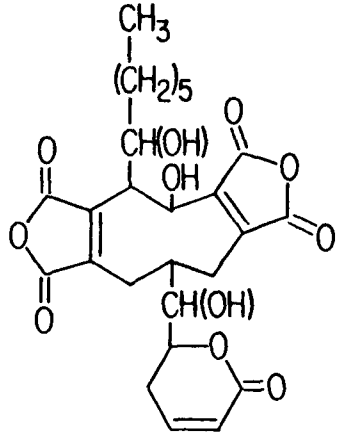
Griseofulvin



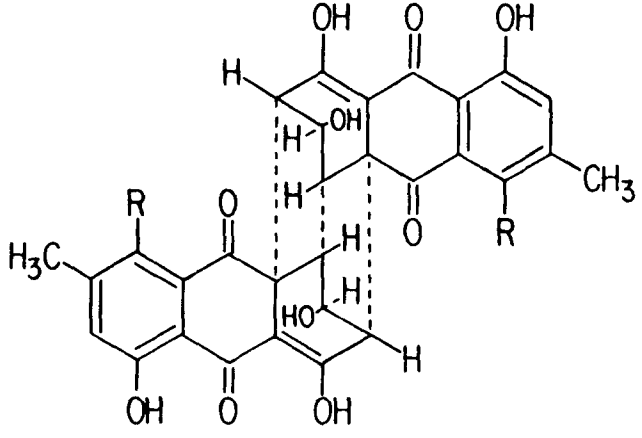
Cyclochlorotine



Islanditoxin



Rubratoxin B



Luteoskyrin R=OH

Rugulosin R=H

5.3.1.2.2 Physicochemical Properties and Biological Effects.

5.3.1.2.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

Penicillium toxins display wide variations in their chemical structure (see Table XII) as well as in physicochemical properties. The ultraviolet, infrared, nuclear magnetic resonance and mass spectral data of many of these compounds have been compiled (9, 10). Some other important physical properties of Penicillium toxins are summarized in Table XIII.

Ochratoxin A is a 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3-R-methyl derivative of isocoumarin linked to the amino group of L-β-phenylalanine. Upon acid or enzymic hydrolysis, L-β-phenylalanine and the isocoumarin acid are formed. Ochratoxin A is quite stable in stored foods, but decomposes readily under fluorescent light (9, 11).

Citrinin (4,6-Dihydro-8-hydroxy-3,4,5-trimethyl-6-exo-3H-2-benzopyran-7-carboxylic acid) resembles structurally the isocoumarin derivative of ochratoxin A. The compound is thermally stable in hexane or ethanol, but is thermally labile in acid or alkaline solution. It is also unstable under fluorescent light and is inactivated by cysteine (9).

PR toxin (7-Acetoxy-5,6-epoxy-3,5,6,7,8,8a-hexahydro-3',8,8a-trimethyl-3-oxospiro[naphthalene-2(1H),2'-oxirane]-3'-carboxaldehyde) has a eremophilane ring system with an acetoxy group, an aldehyde, and an α,β-unsaturated ketone, two epoxides and three methyl groups. The compound reacts with ammonia and free amino acids (12).

Both patulin (a furopyrone) and penicillic acid have a five-membered lactone ring unsaturated in the α,β-position to the carbonyl group. In aqueous solution, penicillic acid is in equilibrium with the corresponding open-chain hexanoic acid (see Table XII). Patulin is stable under acidic conditions or

Table XIII
Physical Properties of Some *Penicillium* Toxins[a]

Toxin[b]	Physical Form	m.p.	Optical Rotation	Solubility
Ochratoxin A	Colorless crystals	89-95°C (benzene)[c] 169°C (xylene)[c]	$[\alpha]_D^{20} = -118^\circ$	Slightly soluble in water; soluble in polar organic solvents
Citrinin	Yellow needles	178°C	$[\alpha]_D^{21} = -27.7^\circ$	Insoluble in water; soluble in dilute alkali, ethanol and dioxane
PR toxin	Colorless crystals	155°C	--	Insoluble in water, dilute acid or alkali; soluble in organic solvents
Patulin	Colorless to white crystals	111°C	Inactive	Soluble in water and polar organic solvents
Penicillic acid	Colorless crystals	87°C	Inactive	Soluble in water, ethanol, ether, benzene and chloroform
Penicillin G	Amorphous white powder		$[\alpha]_D^{25} = +269^\circ$	Sparingly soluble in water; soluble in methanol, ethanol, acetone, ether, chloroform and ethyl acetate
Griseofulvin	Colorless octahedra or rhombs	220°C	$[\alpha]_D^{17} = +370^\circ$	Insoluble in water, petroleum or ether; slightly soluble in ethanol, methanol, acetone, benzene, chloroform, ethyl acetate and acetic acid

Table XIII (continued)

Toxin[b]	Physical Form	m.p.	Optical Rotation	Solubility
Rubratoxin B	Colorless crystals	169°C	$[\alpha]_D^{20} = +67^\circ$	Sparingly soluble in water; soluble in ethanol, ethyl acetate, dioxane and acetone
Luteoskyrin	Yellow, rectangular crystals	287°C	$[\alpha]_D^{25} = -880^\circ$	Insoluble in water; soluble in sodium bicarbonate and most organic solvents
Rugulosin	Yellow, prism-like crystals	290°C	$[\alpha]_D^{25} = +492^\circ$	Insoluble in water; soluble in sodium bicarbonate and most organic solvents
Cyclochlorotine	White needles	251°C	$[\alpha]_D^{16} = -92.9^\circ$	Soluble in water and n-butanol

[a]Summarized from IARC Monographs, Vol. 10, 1976; P.M. Scott, Penicillium Mycotoxins. In "Mycotoxin Fungi, Mycotoxins, Mycotoxicoses, An Encyclopedic Handbook" (T.D. Wyllie and L.G. Morehouse, eds.), Vol. 1, Part 2, Marcel Dekker, New York, 1977, p. 283; The Merck Index, 10th ed., Merck & Co., Rahway, NJ, 1983.

[b]See Table XII for structural formulas.

[c]Solvents used in crystallization.

in chloroform, but is unstable in alkaline solutions or in methanol. The secondary alcohol group of the hemiacetal moiety of patulin may be esterified to form a monoacetate, benzoate or cinnamate. Various derivatives of patulin can also be formed involving the carbonyl group (9, 13). Unlike patulin, penicillic acid is stable under either acid or alkaline conditions. The reaction of penicillic acid with phenylhydrazine or excess diazomethane yields a pyrazoline derivative. Both patulin and penicillic acid react readily with sulfhydryl-containing amino acids or proteins (9, 13).

Penicillin G (benzylpenicillinic acid) consists of a thiazolidine ring linked to a beta-lactam ring, to which a benzyl side chain is attached. Removal of the benzyl side chain chemically or by the action of amidase yields 6-aminopenicillanic acid (see rev. 14).

Griseofulvin (7-Chloro-4:6:2'-trimethoxy-6'-methylgris-2'-en-3:4'-dione) is a polycyclic chlorine-containing compound. Acid hydrolysis of griseofulvin gives griseofulvic acid which, upon further hydrolysis with 0.5 H sodium hydroxide, yields norgriseofulvin and decarboxygriseofulvic acid (15).

Rubratoxin B is a substituted analog of byssochlamic acid in which the ethyl group is replaced by a 6-carbon α,β -unsaturated lactone. Oxidation of rubratoxin B with chromic acid in acetone at $0^{\circ}C$ yields monoketone derivatives. The toxin is stable in sodium bicarbonate (16).

Luteoskyrin is a substituted bis-polyhydroxydihydroanthraquinone. Reaction of leuteoskyrin with 60% sulphuric acid yields islandicin and iridoskyrin. Rugulosin, also an anthraquinone, is chemically related to luteoskyrin. Both toxins can chelate divalent cations such as magnesium and calcium ions (see rev. 17).

Cyclochlorotine is a water-soluble cyclic pentapeptide containing residues of a dichloroproline, α-aminobutyric acid, serine, β-amino-β-phenylbutyric acid, and serine. The compound gives a positive result in biuret test, but is negative in the ninhydrin reaction (cited in 10).

Islanditoxin is a cyclic peptide isomeric with cyclochlorotine. The physicochemical properties of islanditoxin resemble those of cyclochlorotine.

5.3.1.2.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENICITY.

Toxic Effects. The presence of fungi in foods and animal feeds has long been incriminated in outbreaks of human diseases and poisonings of poultry, swine and cattle. The common deleterious effects on farm animals include reduced feed intake, decreased weight gains and lower production of egg and milk. Consumption of large doses of mycotoxins generally results in animal deaths. The LD_{50} values of some Penicillium toxins in laboratory rodents are shown in Table XIV. Among all compounds, the "yellowed rice" toxins cyclochlorotine and islanditoxin are the most potent ones; rubratoxin B is also extremely toxic to the rat and mouse when administered intraperitoneally. In a chick embryotoxicity test, the lethal doses for several Penicillium toxins are: ochratoxin A, 0.1 μg; PR toxin, 0.1 μg; rubratoxin B, 0.1 μg; patulin, 1.0 μg; citrinin, 10 μg; penicillic acid, 10 μg; and griseofulvin, 100 μg (34). As many of these mycotoxins may occur simultaneously in mold-contaminated foods and feeds, the possibility of toxic interaction is receiving increasing attention. A synergistic effect between the acute toxicities of ochratoxin A and citrinin (35-37) and between the acute toxicities of ochratoxin A and penicillic acid (27, 35, 38) in rodents has been reported.

Pathological observations indicate that different organ system may be characteristically affected by particular mycotoxins. Ochratoxin A and

Table XIV
Acute Toxicity of Some *Penicillium* Toxins

Toxin[a]	Species and Route	LD_{50} (mg/kg)	Reference
Ochratoxin A	Rat, oral	28	18
Citrinin	Rat, s.c., i.p.	67	19
	Mouse, oral	110	20
	s.c., i.p.	35	19
	i.v.	38	20
	Rabbit, i.v.	19	19
	Guinea pig, s.c.	37	19
PR toxin	Rat, oral	115	21
	i.v.	8.2	22
	i.p.	11.6	21
	Mouse, oral	72	12
	i.p.	5.8	22
Patulin	Rat, oral	55	23
	s.c.	11	23
	i.p.	10	23
	Mouse, oral	48	24
	s.c.	10	24
	i.p.	7.5	24
	Hamster, oral	31.5	25
	s.c.	23	25
	i.p.	10	25
Penicillic acid	Mouse, oral	600	26
	s.c.	110	26
	i.v.	250	26
	i.p.	70	27
Penicillin G	Mouse, i.v.	168	28
Griseofulvin	Rat, i.v.	400	8
Rubratoxin B	Rat, oral	400	29
	i.p.	0.36	29
	Mouse, oral	400	8
	s.c.	6.8	30
	i.p.	2.6	29
Luteoskyrin	Mouse, oral	221	17
	s.c.	147	17
	i.v.	6.6	17
	i.p.	40.8	17

Table XIV (continued)

Toxin[a]	Species and Route	LD_{50} (mg/kg)	Reference
Rugulosin	Rat, i.p.	44	31
	Mouse, i.p.	55	31
Cyclochlorotine	Mouse, oral	6.55	32
	s.c.	0.48	32
	i.v.	0.34	32
Islanditoxin	Mouse, oral	6.5	Cited in 33
	s.c.	0.47	Cited in 33
	i.v.	0.3	Cited in 33

[a]See Table XII for structural formulas.

citrinin are primarily nephrotoxic, causing kidney enlargement, tubular necrosis and disruption of renal functions in varying animal species (39, 40; rev. 9). These two toxins have been suspected to be the etiologic agents of porcine nephropathy in Denmark (41). There is also epidemiologic evidence suggesting that these two mycotoxins may be involved in the endemic "Balkan nephropathy," a renal disorder of approximately 20,000 people living along the Danube River in Romania, Bulgaria, and Yugoslavia (42, 43).

Chu and associates (44, 45) have studied the relationships between the structure and toxicity of ochratoxin A and its derivatives in ducklings. Whereas the methyl ester and the ethyl ester (ochratoxin C) of ochratoxin A are as toxic as ochratoxin A, the dechlorinated analog (i.e., ochratoxin B) of ochratoxin A, the 4-hydroxylated ochratoxin A (i.e., ochratoxin D) and the hydrolysis products of ochratoxin A (i.e., ochratoxin α) and of ochratoxin B (i.e., ochratoxin β) are much less toxic. On the basis of these findings, Chu and coworkers (44, 45) suggested that the presence of a chlorine atom and/or a phenolic hydroxyl group in the dissociated form are important for the toxicity of these compounds. It was noted that the higher the pK value, the less toxic the compound; for instance, the pK value of ochratoxin A and some of its derivatives are: ochratoxin A, 7.07; ochratoxin C, 7.14; ochratoxin B, 7.95, and ochratoxin α, 11.0.

Rubratoxin B, luteoskyrin, rugulosin, cyclochlorotine and islanditoxin cause pale liver, cirrhosis, necrosis, steatosis, hemorrhage and specific zone lesions in the liver of rats and mice and are best known as hepatotoxins (rev. 9). Although there is no direct evidence for the association of luteoskyrin, cyclochlorotine, rugulosin, islanditoxin and citrinin with the "yellowed rice syndrome" of Japanese who consumed moldy rice, the fungi that produce these toxins have been isolated from the contaminated rice. The symptoms of the

disease mimic those of acute beriberi and are characterized by vomiting, convulsions, ascending paralysis, and respiratory arrest (46).

In the mouse, the liver is also a target organ of griseofulvin, a potent porphyrogenic and antimitotic agent which produces various types of liver damage (47). Like the well-known spindle poison colchicine, griseofulvin inhibits the assembly of microtubule and disrupt the mitotic apparatus of the cell (e.g., 48). The antimitotic effect is due to the interaction of the toxin with the sulfhydryl group of either tubulin (49) or other microtubule-associated proteins (50, 51).

Patulin is classified principally as a neurotoxin (8, 52). However, the compound also produces pulmonary edema, hepatic necrosis and gastrointestinal hyperaemia in the rat, mouse and hamster (23-25, 53). Oral administration to humans has been reported to result in nausea and stomach irritation. Application of ointment containing 1% patulin to the human skin caused edema (cited in 9). Studies in mice showed that the toxicity of patulin is enhanced by treatment with SKF-525A, indicating that the parent compound, not a metabolite, is the toxic form of this mycotoxin (53). On the other hand, penicillic acid, which causes a generalized necrosis of hepatocytes and various histopathological lesions of the kidney and thyroid gland in mice, is possibly be metabolized into a more toxic intermediate(s), since the acute toxicity of penicillic acid in the mouse is increased by pentabarbital and 3-methylcholanthrene pretreatment but decreased by SKF-525A (54).

PR toxin causes edema in the lung and direct damage to the liver, kidney and heart in mice, rats and cats (12, 22). Comparison between the chemical structures and the biological properties of some eremophilane compounds related to PR toxin suggested to Moule et al. (55) that the aldehyde group in

position 12 rather than the two epoxide moieties, or the acetyl group on the eremophilane ring is associated with the toxic effects. This is supported by the finding that PR imine, an analog of PR toxin without the aldehyde group in 12, is considerably less toxic in the mouse than the parent compound (12, 55).

Penicillin G possesses potent antimicrobial activity against gram-positive and gram-negative cocci, gram-positive bacilli, spirochetes, actinomycetes and psittacosis virus. In some individuals receiving sodium penicillin G for treatment of infectious diseases, local and generalized allergic reactions, convulsions, bronchospasm and nephropathy may occur (see rev. 14).

Mutagenic Effects. The mutagenicity of Penicillium toxins has been tested in Salmonella typhimurium, Bacillus subtilis, Escherichia coli, Aspergillus nidulans, Saccharomyces cerevisiae and several mammalian systems (Table XV).

According to the data from the studies using S. typhimurium strains TA98, TA100, TA1535 and TA1538, only PR toxin shows positive results in one study (61) using strain TA98 with the addition of S-9 mix; all other toxins were negative with and without S-9 mix (8, 56, 57, 60, 61, 64, 66, 75, 77, 83). However, considerable concern has been voiced regarding the sensitivity and adequacy of such screening systems for these toxins since these tester strains detect only reverse mutations representing only limited types of genetic alterations. Indeed, Stark et al. (83) showed the mutagenicity of rugulosin and a photoproduct of luteoskyrin (lumiluteoskyrin) in S. typhimurium strain TM677 which detects forward mutations. The mutagenesis assay was carried out in suspension at low concentrations for long exposure periods. Addition of rat liver microsomes to the assay system diminished the mutagenicity. In 1982, a new Salmonella tester strain, TA97, was developed to replace strain

Table XV

Mutagenicity of Some *Penicillium* Toxins

Toxin[a]	*Salmonella typhimurium*[b]	*Bacillus subtilis*	*Saccharomyces cerevisiae*	Chromosomal Aberrations	Other Tests
Ochratoxin A	- (56,57)[c]	- (58)	- (56)	- (59)	n.t.
Citrinin	- (56,57,60,61)	+ (58)	- (56)	+ (62)	-[g] (62)
PR toxin	+ (61,63)[d] - (64)	+ (58)	+ (65)	n.t.	-[g] (64)
Patulin	- (56-59,66)	+ (58)	+ (67) - (56)	+ (59,68-70)	+[g] (71) -[g] (68) -[h] (72,73)
Penicillic acid	- (57,60,61)	+ (58)	- (56)	+ (59,74)	n.t.
Penicillin G	- (75)	- (76)	n.t.	n.t.	+[k] (76)
Griseofulvin	- (56,57,61,77,78)	- (58,76)	- (56)	- (77)	-[h] (79) +[i] (80) +[j] (78)
Rubratoxin B	- (56,60)	- (58)	- (56)	+ (81)	+[h] (82)
Luteoskyrin	- (57,61,83)	+ (58)	+ (84)	- (59)	n.t.
Rugulosin	+[e] (83) - (61,83)	+ (58)	+ (84)	n.t.	n.t.
Cyclochlorotine	- (8)	n.t.[f]	n.t.	n.t.	n.t.

[a] See Table XII for structural formulas.

[b] Strains TA98, TA100, TA1535, TA1537 and/or TA1538.

[c] "+" = positive; "-" = negative; numbers in parenthesis are references.

[d] Strains TA97, TA98.

[e] Strain TM677.

[f] n.t. = not tested.

[g] Sister-chromatid exchange assay.

[h] Mouse dominant lethal assay.

[i] *Aspergillus nidulans*.

[j] Sperm abnormality assay in mice.

[k] *Escherichia coli*.

TA1537 for the detection of frameshift mutagens (63). The mutagenicity of PR toxin was again demonstrated in this more sensitive strain (63). Other Penicillium toxins have not been tested for their mutagenic properties in strain TM677 or strain TA97 of S. typhimurium.

When the genotoxicity of Penicillium toxins was studied in the rec assay in the recombination-deficient mutant of Bacillus subtilis M45 (rec-) and in the parent strain H17 (rec+), positive results were found with citrinin, PR toxin, patulin, penicillic acid, luteoskyrin and rugulosin (58). Penicillin G (76), ochratoxin A, griseofulvin and rubratoxin B (58) were not mutagenic. The latter three compounds, as well as citrinin, patulin and penicillic acid were also not mutagenic in Saccharomyces cerevisiae strain D3 (56). Studies of Wei et al. (65), on the other hand, showed that PR toxin is a direct acting mutagen toward S. cerevisiae strains D4 and D7, causing reverse mutation, gene conversion and mitotic crossing-over without metabolic activation. In agreement with the toxicity results reported by Moule et al. (55), structure-mutagenicity relationship analysis revealed that the aldehyde and the keto groups but not the two epoxide moieties play the key role in the genetic activity of PR toxin. Patulin was reported to be mutagenic in an extrachromosomal mutation system of a haploid strain of S. cerevisiae (67). Luteoskyrin and rugulosin, at low concentrations, induced a high frequency of mutations in a respiratory-deficient mutant strain of S. cerevisiae (84). Studies using the Escherichia coli DNA-repair assay system showed that penicillin G is mutagenic in the absence of microsomal activation (76).

In accord with the negative results obtained in some microbial assay systems, which detect point mutations, cytogenetic studies showed that ochratoxin A (59) and griseofulvin (77) had little effects on the incidence of DNA single-strand breaks and chromosome aberrations in mouse cells. Also, treat-

ment of somatic or sperm cells of the mouse with luteoskyrin did not produce any increase in the rate of chromosomal aberrations (59). However, in the experiments of Kappas and Georgopoulos (80), low concentrations of griseofulvin caused increased frequencies of somatic segregation due to chromosome nondisjunction in a diploid strain of Aspergillus nidulans. Data obtained from the sperm abnormality assay of the mouse also showed that griseofulvin is mutagenic (78). Citrinin (62), patulin (59, 68-70), penicillic acid (59, 74) and rubratoxin B (81) have all been demonstrated to be clastogenic in cells of the mouse, hamster or humans. Whereas citrinin (62), patulin (68) and PR toxin (64) are inactive in the sister-chromatid exchange (SCE) assay in Chinese hamster V79 cells, patulin induces significantly elevated frequency of SCE in human lymphocytes (71). Rubratoxin B (82) but not patulin (72) or griseofulvin (79) showed any mutagenic effects in the mouse dominant lethal assay. The result is also negative for patulin in a dominant lethal assay in rats (73). The structural requirement for the dominant lethal effect of rubratoxin B is the α,β-unsaturated lactone ring (82).

Teratogenic Effects. Ochratoxin A, rubratoxin B, griseofulvin, PR toxin and patulin have all been demonstrated to be embryotoxic and teratogenic in experimental animals.

Exposure of pregnant mice during early stage of gestation (days 8 and 9) to ochratoxin A resulted in increased prenatal mortality and a variety of gross and skeletal abnormalities. The major abnormalities are cranio-facial cleft associated with exencephaly and open eyelid, and skeletal defects involving ribs and vertebrae (85). When mice were exposed to the toxin during the 15th, 16th and 17th day of gestation, significant developmental delay was noted in the pups as indicated by performance in several behavioral tests (86). Teratogenic effects similar to those in mice were found in fetuses from

pregnant rat given low doses (0.25, 0.50 or 0.75 mg/kg) of ochratoxin A by gavage on day 20 of gestation (87). At doses higher than 1 mg/kg, ochratoxin A was embryocidal in the rat (87-89). Golden hamsters are more resistant to the fetotoxic effects of ochratoxin A. The toxin is also highly teratogenic in this species, since high incidence of malformations such as micrognathia, hydrocephalus, micromelia, and heart defects occurred in offspring of pregnant golden hamsters injected intraperitoneally with 2.5-20 mg/kg ochratoxin A on gestation day 7, 8, 9 or 10. The highest dose (20 mg/kg) caused increased prenatal mortality when given on day 7, 8 or 9 of gestation (90). Ochratoxin A also induces embryotoxic and teratogenic effects in chicken. Injection of ochratoxin A (0.5-7 μg/egg) into embryonating eggs resulted in malformations including short and twisted limbs and neck, microphthalmia, exencephaly, everted viscera, and decreased length of survival and body size of the chicken (91).

Like ochratoxin A, rubratoxin B is also teratogenic and induces similar abnormalities in chick embryos (92). Intraperitoneal administration of rubratoxin B (0.4-1.5 mg/kg) to mice resulted in a dose-related increase in early fetal deaths as well as in the incidences of fetal defects (82, 93, 94). The most striking developmental defects caused by rubratoxin B in the mouse are exencephaly, malformed pinnae and jaws, umbilical hernia and "open eye" (93). In structure-activity relationship studies it was found that saturation of the α,β-unsaturated lactone ring in the molecule abolishes teratogenicity (82).

Klein and Beall (95) administered 125-1,500 mg/kg of griseofulvin orally to groups of pregnant rats during organogenesis. Increased frequency of skeletal abnormalities and decreased pre- and postnatal survival rates were observed in the offspring of dams treated with high doses of griseofulvin

(1,250 and 1,500 mg/kg). Scott et al. (96) reported multiple congenital malformations in kittens of three cats given oral doses of 500 or 1,000 mg griseofulvin at weekly intervals during pregnancy. In a chick embryotoxicity screening test, embryonic death and abnormal development of the caudal trunk were observed after administration of 100 ug and 10 ug of griseofulvin, respectively (34). The corresponding doses with PR toxin to exert such effects in this test were merely 0.1 μg and 0.01 μg. Griseofulvin causes embryonic death and abnormalities in newborn animals by interfering with the formation of cell organelles, especially with the mitotic spindle (see Section 5.3.1.2.2.2).

Treatment of pregnant mice with 10-40 mg/kg citrinin (97) or 30-90 mg/kg penicillic acid (98) caused a significant increase in prenatal mortality of the offspring at the highest doses, but no malformations were noted in the surviving fetus. There were no defects in the fetuses of mice (99) or rabbits (100) given daily doses of 30-300 mg/kg (mice) or 10-100 mg/kg (rabbits) penicillin G during pregnancy. Similarly, no evidence of teratogenicity was found in the mouse (72) or rat (73, 101) administered patulin in the range of 1.5-15 mg/kg body weight. However, Ceigler and associates (102) observed various skeletal abnormalities in chick embryos treated with patulin. Upon incubation of human placenta with patulin, Fuk-Holmberg (101) noted sharp increases in the activities of malate dehydrogenase and RNase. These effects of patulin on placental enzymes were interpreted by the author as indicating physiological and functional disorders in the tissue.

5.3.1.2.3 Carcinogenicity and Structure-Activity Relationships

The carcinogenicity of Penicillium toxins were first suggested by the observations that chronic ingestion by mice or rats of diets containing cul-

tures of molds (producing these toxins) resulted in the induction of neoplasms. In Swiss mice fed a rice culture of P. viridicatum (the fungus that produces ochratoxin A, citrinin, penicillic acid and griseofulvin) in the diet (7.5%), a 57% higher incidence of pulmonary tumors was observed than in the controls (103). Similarly, administration to 30 rats of diets containing rice cultures of P. islandicum (which produces luteoskyrin, cyclochlorotine and islanditoxin) led to the development of hepatomas in 5 animals (104).

So far, only a small number of Penicillium toxins has been studied adequately for carcinogenicity in long-term experiments, due probably to their potent toxicity and to the limited production of these metabolites by fungi. The evidence is substantial for the carcinogenicity of ochratoxin A, griseofulvin, luteoskyrin and cyclochlorotine in experimental animals. Results from preliminary studies also point to a carcinogenic potential of citrinin, PR toxin and rugulosin. Although carcinogenicity has not been demonstrated by other routes of administration, patulin, penicillic acid, and penicillin G are tumorigenic in rats following subcutaneous injection. Islanditoxin, a cyclic peptide isomeric to cyclochlorotine, was described as a carcinogenic mycotoxin (105). The carcinogenicity studies on Penicillium toxins are summarized in Table XVI. It is interesting to note that ochratoxin A, patulin and penicillic acid all contain a lactone moiety in their molecules. Like aflatoxin and sterigmatocin, citrinin, griseofulvin, luteoskyrin and rugulosin are biosynthesized by the acetate-malonate pathway (124; rev. 9) and all contain a phenol or quinone moiety (see Table XII).

In general, the organ or tissues which are susceptible to toxic effects of these toxins are also the targets for tumor induction. The hepatotoxins luteoskyrin, rugulosin, cyclochlorotine and griseofulvin all induce liver neoplasms whereas the nephrotoxin citrinin is carcinogenic toward the kidney

Table XVI
Carcinogenicity of *Penicillium* Toxins

Toxin[a]	Species and Strain	Principal Organs Affected and Route	Reference
Ochratoxin A	Mouse, ddY	Liver, kidney; oral	106
	Rat, F344	Liver[b]; oral	107
	Rat, Wistar	None; oral, s.c.	108
PR toxin	Rat, albino	Neck, uterus; oral	109
Citrinin	Rat, F344	Kidney; oral	110
Patulin	Rat, --[c]	Local sarcoma; s.c.	111
	Rat, Sprague-Dawley	None; oral	112
Penicillic acid	Rat, --[c]	Local sarcoma; s.c.	111, 113, 114
	Mouse, --[c]	Local sarcoma; s.c.	114
Penicillin G (sodium salt)	Rat, --[c]	Local sarcoma; s.c.	111, 113
Griseofulvin	Mouse, Alderley Park	Liver; oral	115
	Mouse, Charles River albino	Liver; oral	116
	Mouse, Swiss	Liver; s.c.	117, 118
	Mouse, white, nunu	Liver; --[c]	119
	Mouse, Swiss	Liver; oral	120
	Rat, MRC-Wistar	Thyroid; oral	120
	Rat, Wistar	None; i.p.	121
	Rat, guinea pig, rabbit, --[c]	None; oral	115
	Hamster, Syrian	None; oral	120
Rubratoxin B	Rat, Fischer	None; oral	29
Luteoskyrin	Mouse, ddNi, ddN	Liver; oral	32
	Mouse, DDD	Liver; oral	122
Rugulosin	Mouse, ddYS	Liver; oral	123
	Rat, F344	Liver[b]; oral	107
Cyclochlorotine	Mouse, ddNi, ddN	Liver; oral	32

[a] See Table XII for structural formulas.

[b] Based on the initiating and promoting activities in liver carcinogenesis.

[c] Strain/route of administration not reported.

(see Table XVI). However, studies of griseofulvin and PR toxin have also revealed tumor induction in the thyroid, uterus and/or neck of animals, indicating that several target tissues are affected by Penicillium toxins. The histogenesis and ultrastructural changes of liver tumor cells following treatment with hepatotoxic mycotoxins of this class have been fully described and were shown to be similar to findings in human hepatomas (125).

Ochratoxin A. In 1971, a pilot study on the carcinogenicity of ochratoxin A was conducted in rainbow trout (Salmo garidneri). Hepatomas were noted in rainbow trout fed ochratoxin A at the level of 20 ppb together with the cocarcinogen, sterculic acid. However, no tumors were found when ochratoxin A was fed alone at the levels of 16, 32 or 64 ppb for 8 months (126).

Ochratoxin A is a fairly strong carcinogen toward the liver and kidney of the mouse. Feeding 40 ppm ochratoxin A in the diet for 44 weeks produced 8 hepatic cell tumors, 5 renal cell tumors, and 18 cyctic adenomas of the kidney in 19 ddY mice. Whereas dosing with aflatoxin B_1 (a single dose of 20 mg/kg) alone elicited only 2 hepatic cell tumors and no renal cell tumors in 18 mice, administration of aflatoxin B_1 followed by ochratoxin feeding (40 ppm, 44 weeks) induced 15 hepatic and 3 renal cell tumors in 20 mice, indicating a synergistic effect of aflatoxin B_1 on hepatocarcinogenesis of ochratoxin (106).

In the rat (Wistar-derived), Purchase and Van der Watt (108) failed to induce a significant incidence of tumors by administering either 2.5 mg/kg ochratoxin A subcutaneously twice weekly for 17.5 weeks or 0.3 mg ochratoxin A orally 5 times/week for 50 weeks. They have noted a hamartoma of the kidney in one of the ten rats which received ochratoxin A orally. Using F344 rats, Imaida et al. (107) investigated initiation and promotion by ochratoxin A in

liver carcinogenesis. In these bioassays, N-2-fluorenylacetamide (200 ppm in diet) was used as an initiator (or a promotor) and ochratoxin A was given to the rats at a dietary level of 50 ppm for 6 weeks during the initiation stage (or the promotion stage). Ochratoxin A displayed both initiating and promoting activity and was termed a hepatocarcinogen (107).

PR Toxin. The carcinogenic potential of PR toxin in the rat has been investigated by Polonelli et al. (109). A group of 10 albino weanling rats of both sexes was given 200 ppm PR toxin in drinking water for 52 days. About 13 months after the treatment, a squamous epithelioma developed in the neck region of one rat and after about 3 more months of observation, an uterine sarcoma was detected in another rat. None of the 10 matched control animals developed any tumors during the same course of the study. Although the tumor incidences are not statistically significant and further studies are needed, the development of these tumors, particularly the squamous epithelioma in the neck, was considered treatment-related on the basis of historical data showing that spontaneous tumor of this type is rare in the rat.

Citrinin. Early investigations have demonstrated both the tumorigenesis initiating and promoting activity of critinin in the rat. Imaida and coworkers (107) showed that administration of citrinin to F344 rats in the initiating stage and of N-2-fluorenylacetamide in the promoting stage significantly increased the number and area of liver hyperplastic nodules as compared with those in the control group (which did not receive citrinin pretreatment). Whereas N-(3,5-dichlorophenyl)succinimide (NDPS) or citrinin alone did not induce kidney tumors in Sprague-Dawley rat, feeding of NDPS for 8 weeks followed with citrinin (0.02%) for 20 weeks resulted in renal cell tumor in 4 of 18 rats (127). Moreover, the kidney tumor incidence in rats treated with

citrinin following dimethylnitrosamine (DMN) was much higher than the incidence in rats treated with DMN alone (127).

Arai and Hibino (110) were the first to present direct evidence showing that citrinin is indeed carcinogenic, producing kidney adenomas in the rat. Among 48 male F344 rats given 0.1% citrinin in the diet for up to 80 weeks, 35 (72.9%) developed renal epithelial tumors (not found in the controls).

Patulin and Penicillic acid. Interest in the carcinogenicity studies of patulin and penicillic acid arose in the early 1960's when F. Dickens and H.E.H Jones of England drew attention to the possible carcinogenic activity of chemicals having a lactone ring in the molecule (see Vol. IIIA, Section 5.2.1.1.6 on lactones). Patulin and penicillic acid, both having a five-membered lactone ring and an α,β-unsaturated bond, are clearly carcinogenic by repeated injections into rats (111, 113, 114). When 0.2-2.0 mg patulin was injected twice weekly into subcutaneous sites in the flank of 2-month-old male rats, local sarcomas arose in six of eight rats that survived for 1 year. Penicillic acid, at doses of 1 mg in arachis oil, gave rise to highly malignant tumors at the injection sites in all four rats that survived 64 weeks of treatment (111). Later experiments showed that a dose of penicillic acid as low as 0.1 mg is sarcomatogenic in one of four animals surviving for 94 weeks (113, 114). Subcutaneous injections of aqueous solution of penicillic acid (2 mg/0.5 ml water) also produced sarcomas in 4 of 5 surviving rats indicating that the oil vehicle dose not play a significant role in the tumorigenicity (113). Similarly, local sarcomas occurred in 6 of 19 mice receiving subcutaneous doses of 0.2 mg penicillic acid twice weekly for 65 weeks (114).

Patulin is not carcinogenic in animals by oral administration. A total oral dose of 358 mg patulin/kg given over a period of 64 weeks produced no tumors in 50 female Sprague-Dawley rats (112).

Penicillin G. Dickens and Jones (111, 113) have investigated the carcinogenic potential of penicillin G in rats by subcutaneous injection for 65 weeks. In an early study, tumors at the injection site were observed in 2 of 8 animals that survived for at least 59 weeks (111). In a subsequent study, 5 of 11 animals developed fibrosarcomas after 108 weeks; one of the tumors was highly malignant and was transplantable (113). Interestingly, 6-aminopenicillanic acid, a penicillin analog which lacks the benzyl side chain, is a much less potent carcinogen under the same study conditions (114). The carcinogenic action of other members of the penicillin group (see rev. 14), which contain various side chains, has not been tested.

Griseofulvin. The mouse is highly susceptible to the hepatocarcinogenicity of griseofulvin. The presence of hyperplastic nodules is readily seen in the livers of Swiss mice following griseofulvin administration (2.5% in the diet) for 6 to 8 months (128, 129). High incidence of hepatomas have been repeatedly reported in various strains of mice following on prolonged administration of griseofulvin either orally (115, 116, 119, 120) or parenterally (117, 118). Among 13 Alderly-Park strain mice which ingested 1% griseofulvin in the diet for 435 days, 10 were found to bear multiple hepatomas; 5 of 20 mice fed a 0.5% diet also developed tumors in the liver (115). Rustia and Shubik (120) reported that the liver tumor incidence show a dose-response in Swiss mice given 0, 0.3, 1.5 and 3.0% griseofulvin in the diet daily (during alternating 5-week periods for life). At the 3.0% dietary level, the incidences of hepatomas in male and female mice were 83.3% and 87.0%, respectively; the corresponding liver tumor incidences at the 1.5% dietary level were 68.0% and 53.6%; no significant liver tumors were found in the mice at the 0.3% dose level. In addition to nodular hyperplasia, neoplasms of the liver have also occurred in mice of "nunu" strain after 12-14

months of griseofulvin treatment (119). DeMatteis and coworkers (116) noted a marked sex difference in hepatoma incidence of Charles River albino mice which received 1% griseofulvin orally for 12-16 months; the male animals showed a higher incidence as well as multiplicity of these tumors than the females. Significant incidence of hepatomas was also found in Swiss mice subcutaneously injected a total dose of 3 mg griseofulvin at birth and infancy (117, 118). Moreover, cocarcinogenic and promoting effects upon skin tumorigenesis in Swiss-Webster mice were noted when low doses (10-15 mg/kg) of griseofulvin were administered orally before, during or following topical applications of methylcholanthrene (130). In agreement with the tumorigenesis-promoting activity of griseofulvin in the mouse, in vitro transformation of Swiss 3T3 cells infected with tsA mutants of the virus SV40 was enhanced following exposure to either griseofulvin or to the potent tumorigenesis promotor, phorbol ester (131).

In MRC-Wistar rats oral administration of griseofulvin to groups of 30 males and females life resulted in significant incidence of thyroid tumors in a dose-response manner at dietary levels of 0.2, 1.0 and 2.0% (120). However, groups of Syrian golden hamsters given 0.3, 1.5 or 3.0% griseofulvin in the diet for the whole lifespan did not develop tumors (120). Other studies using rats (115, 121), guinea pigs or rabbits (115) yielded little information on the carcinogenicity of griseofulvin. The failure of several experiments (115, 121) to elicit tumors in these species appears to have resulted from the too short exposure periods and/or the small number of animals used.

Rubratoxin B. The carcinogenic potential of rubratoxin B has only been explored by Wogan and coworkers (29) during a chronic toxicity study of rubratoxin B in the rat. Groups of 10-20 Fischer rats of both sexes were intubated with rubratoxin B at a dose of 5 or 10 mg/kg 3 times weekly for 60 weeks. No

evidence of preneoplastic or neoplastic lesions was observed in animals killed after 82-87 weeks. Also, there was no enhancement of the carcinogenic activity of aflatoxin B_1 by rubratoxin B when rats were exposed simultaneously to both toxins.

Nonetheless, in view of the reported mutagenicity of the compound (see Section 5.3.1.2.2) and the presence in the molecule of reactive carbonyl groups and ethylenic double bonds, further exploration on the possible carcinogenic activity of rubratoxin B in other assay systems appears desirable.

Luteoskyrin and Cyclochlorotine. Long-term feeding studies in the mouse have shown that these two mycotoxins exhibit similar chronic effects and are both carcinogenic toward the liver. In a series of experiments conducted by Uraguchi and coworkers (32), significant incidences of benign and malignant liver tumors were induced in a dose-response manner in groups of 8-30 ddNi and ddN strain mice fed luteoskyrin (0, 50, 150 or 500 ug/day) or cyclochlorotine (0, 40 or 60 μg/day) for up to 2 years. Of 26 DDD strain mice given daily doses of 160 ug luteoskyrin in the diet for 328 days, 17 were found by Ueno et al. (122) to bear hepatomas of various histological types.

Rugulosin. In a preliminary study in which groups of 16 DdYS male mice were administered daily doses of 12 or 25 mg/kg rugulosin in the diet for over 800 days, 4 animals bearing hyperplastic nodules composed of hepatocytes were found in both groups. In addition, one animal bearing a hepatocellular adenoma was found in the high-dose group. None of the 14 control mice had such lesions in the liver. These results led the authors (123) to suggest that rugulosin is possibly a weak hepatocarcinogen in mice with a potency about one tenth that of luteoskyrin. The carcinogenic potential of rugulosin was supported by a study demonstrating that rugulosin possesses initiating as well as promoting activity in hepatocarcinogenesis in the rat (107).

5.3.1.2.4 Metabolism and Possible Mechanism of Action

Information regarding the metabolism of these mycotoxins is scanty and their mechanisms of carcinogenic action is unknown. Previously, we have discussed the reaction mechanisms of carcinogenic β-lactones with nucleophilic centers (Section 5.2.1.1.7, Vol. IIIA). Similar reactions probably also occur between nucleophiles and carcinogenic mycotoxins of this group since they all (with the exception of cyclochlorotine and islanditoxin) possess one or more lactone or ketone carbonyl groups with α,β-unsaturation which, upon metabolic oxidation, can be transformed into alkylating intermediates (e.g., epoxides). Consistent with results of mutagenicity studies, patulin, penicillic acid, rubratoxin B and luteoskyrin all form adducts with DNA and/or chromatin. The interaction between the sulfhydryl and amino group of proteins, on one hand, and patulin, penicillic acid, ochratoxin A, luteoskyrin and rubratoxin B, on the other hand, is well documented. Such reactions have been postulated to account for a wide range of their biological and biochemical activities including alteration of carbohydrate and lipid metabolism, inhibition of protein and nucleic acids synthesis and impairment of cell respiration, membrane transport, etc. It is possible that one or a constellation of these activities acting in a concerted manner may bring about permanent structural and functional changes in the cells, leading eventually to neoplasia.

Ochratoxin A. The metabolism of ochratoxin A has been studied in several animal species including the rat (132-134), the pig (135) and the cow (cited in ref. 9). After a single intraperitoneal injection into rats, ochratoxin A was detected in the serum, liver and kidney (132, 134). Part of ochratoxin A was metabolized to ochratoxin α (the isocoumarin acid derived from the loss of the phenylalanine moiety of ochratoxin A) and 4-hydroxyochratoxin A which,

along with the unchanged toxin, were excreted primarily in the urine. Ochratoxin α is also the major metabolite in pigs (135), cows (cited in ref. 9) or rats (133) dosed orally with ochratoxin A. Although ochratoxin α is much less toxic than ochratoxin A toward chick embryos (136), ducklings (44) and rainbow trout (137), it is more inhibitory than the parent compound to the respiration of isolated rat liver mitochondria (138).

Ochratoxin A interacts strongly with serum albumin both in vitro (139) and in vivo (134). There is no evidence as yet for the binding of ochratoxin A to nucleic acids. Treatment of rats with ochratoxin A results in significant depletion of liver glycogen and decrease of the activities of hepatic enzymes such as cyclic AMP-protein kinase, carboxypeptidase and phenylalanine t-RNA synthetase, etc. In certain bacteria ochratoxin A is a potent inhibitor of protein and RNA synthesis (see 140).

Citrinin. In rats (141), rabbits or dogs (142) citrinin is rapidly absorbed and excreted. Peak citrinin levels in the serum, liver and kidney were attained within 30 minutes after parenteral administration. At a non-nephrotoxic dose of 3 mg/kg, about 74% of the administered citrinin was excreted, mostly unchanged, in the urine of rats by 24 hours after administration. However, in rats, rabbits, and dogs, which received higher doses citrinin, a much smaller percentage of the toxin or its metabolites were detected in the urine. The metabolites of citrinin have not been identified as yet. Some of its metabolites are suspected to be dihydrocitrinins (142).

Disturbance of carbohydrate metabolism (143) and inhibition of proteolysis in kidney phagolysomes (144) were noted in mice treated with citrinin.

PR Toxin. In addition to mutagenicity data indicating the genotoxicity of PR toxin, macromolecular binding studies have shown that the compound binds significantly to RNA, DNA and protein in cultured cells as well as in isolated nuclei (145). Moreover, Moule et al. (145) have shown that the toxin cross-links between DNA and protein in the chromatin. The authors implicated exclusively the aldehyde group in the PR toxin molecule, which would form a methylene bridge between an amino groups in DNA and a functional group in chromatin protein. However, the present writers feel that cross-linking via the reactive epoxide groupings in the PR toxin molecule cannot be discounted. PR toxin has also been shown to impair liver cell metabolism by inhibiting macromolecule synthesis (146).

Patulin and Penicillic acid. Both patulin and penicillic acid are rapidly absorbed in the gastrointestinal tract. In metabolic studies with [^{14}C]-patulin (147) or [^{14}C]-penicillic acid (148) in rats, most of the [^{14}C]-radioactivity was recovered from urine and feces within 24 hours after dosing. However, appreciable levels of radioactivity remained in the red blood cells, liver, kidney and lung for up to 7 days. Significant amount of radioactivity becomes bound to DNA, RNA and protein in the liver cells following administration of [^{14}C]-penicillic acid to rats (148). The metabolites of patulin and penicillic acid have not been identified.

Patulin and penicillic acid are potent inhibitors of polymerases (149), ATPases (150, 151) and various thiol enzymes (152, 153) in vitro. The effects are presumed to be due to interaction of the toxins with sulfhydryl and amino groups of these enzymes. Indeed, patulin and penicillic acid are known to readily combine with sulfhydryl compounds to form S-alkylated adducts by interaction of the nucleophilic sulfhydryl group with the double bond(s) (154, 155). Penicillic acid also reacts, albeit at a slow rate, with lysine,

arginine and histidine, at pH 7.0 (154). The inactivation of polymerases, ATPases, and thiol enzymes probably accounts for the inhibitory effects of patulin and penicillic acid on macromolecular synthesis (156), active membrane transport (157, 158) and cellular respiration (159). Although the relationship between these biochemical effects and the mechanism of their carcinogenic action is not clear, investigation of the reaction of unsaturated γ-lactones with cysteine has shown that S-alkylated adducts are formed only with carcinogenic lactones but not with noncarcinogenic lactones (160).

Penicillin G. In humans, about 30% of an oral dose of penicillin G is absorbed in the small intestine, while a large quantity remains unabsorbed and passes into the colon. The absorbed penicillin G is widely distributed in the body fluids and tissues. Significant levels of penicillin G can be found in the liver, bile, kidney and plasma. The compound is excreted mainly through the kidney and bile; a small amount is excreted in milk and saliva. One of the urinary metabolites has been identified as 6-aminopenicillanic acid (see rev. 14), which is a less potent carcinogen than penicillin G. Since the benzyl side chain is absent in 6-aminopenicillanic acid, Dickens and Jones (114) speculated that the side chain might contribute to the carcinogenic action of penicillin G. On the other hand, penicillins were suggested to act as alkylating or acylating agents (see rev. 161) by way of the probable reaction mechanisms (shown in Fig. 4), which would be influenced little if at all by the benzyl side chain.

Griseofulvin. The metabolic fate of griseofulvin in mammalian species have been critically reviewed by Lin and Symchowicz (162). In the mouse, rat,

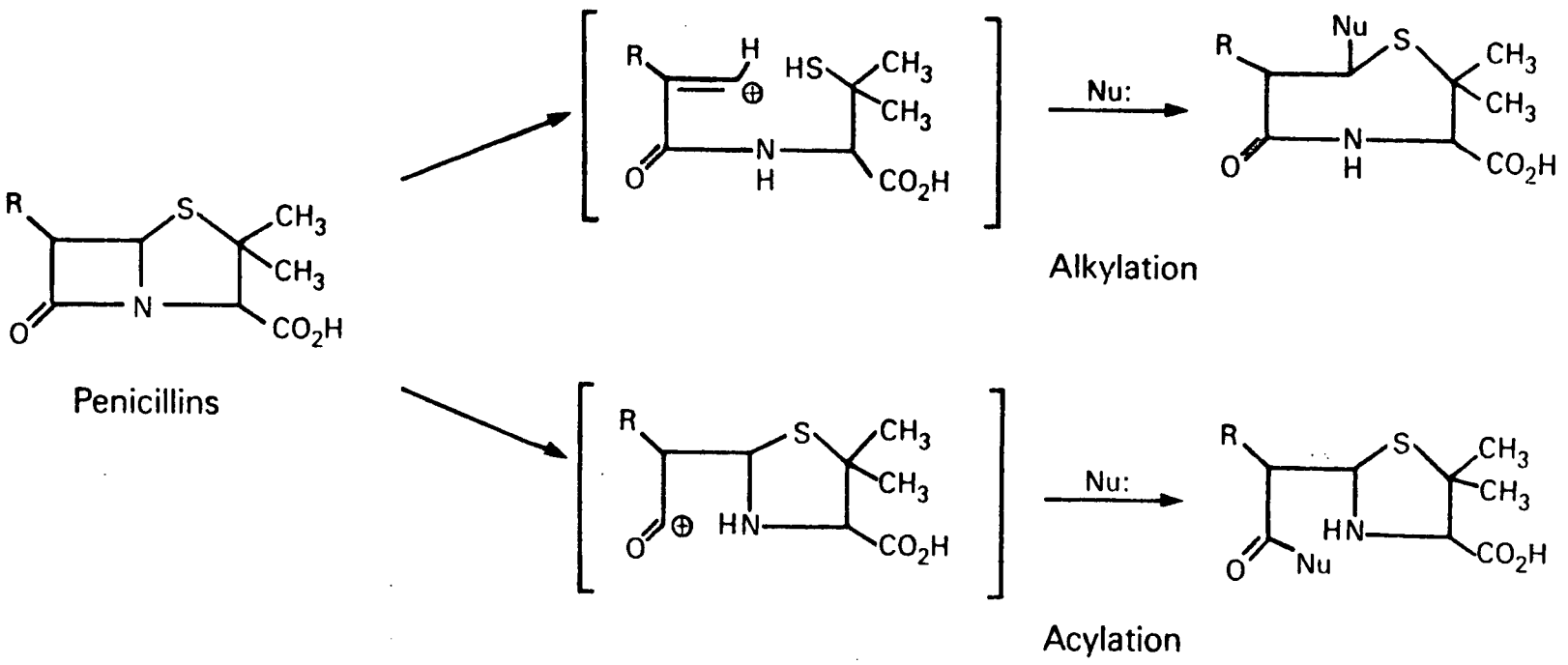


Fig. 4. Probable reaction mechanisms of alkylation and acylation by penicillins.

rabbit, dog and human, the rate of absorption is rapid and most of the compound is excreted in the urine as metabolites. Studies in the rat showed that the highest level of griseofulvin occurs in the liver after oral administration and in the lung following subcutaneous injection. In the mouse and rat, both 4-desmethylgriseofulvin and 6-desmethylgriseofulvin are the major metabolites; in rabbits, dogs and humans, on the other hand, the only major metabolite is 6-desmethylgriseofulvin. In the rabbit, griseofulvin is also metabolized to 3-chloro-4,5-dimethoxysalicylic acid (163). Several unidentified additional metabolites of griseofulvin have been found in human urine (164).

In the mouse liver, griseofulvin induces the proliferation of the smooth endoplasmic reticulum, it increases the amount of NADPH-cytochrome c reductase, and stimulates the metabolism of other exogenous chemicals (165).

Rubratoxin B. Hayes (166) studied the distribution and excretion patterns of rubratoxin B in mice and rats. During the first 24-hour period following administration of [^{14}C]-rubratoxin B (0.05 mg/kg, i.p.) to mice and rats, 30-40% of the radioactivity was excreted through respiration as CO_2, 6-9% was recovered in the urine and a small amount was found in the feces. In both species, the concentration of radioactive substances was higher in the liver than in other tissues. In the liver, radioactivity was about 54-80% in the cytosol, 14-25% in the mitochondrial fraction, 7-12% in the nuclear fraction and 3-10% in the microsomal fraction. Consistent with the findings of the subcellular distribution studies, rubratoxin B inhibits oxygen uptake, ATPase activity and electron transport in liver mitochondria (167), binds to DNA (cited in rev. 168) and causes disaggregation of polysomes (169).

Luteoskyrin and Rugulosin. Pharmacokinetic studies in the mouse (170, 171) showed slow uptake and slow excretion of ^{3}H-luteoskyrin following subcutaneous or oral administration. During the 18 days after dosing, only 19% and 6% of the administered ^{3}H-luteoskyrin were excreted in the feces and urine, respectively. The liver accumulated 83-94% of the total organ localized radioactivity; only a minute quantity of radioactivity was present in the lung, kidney and spleen (170). The ^{3}H-luteoskyrin level in the liver of male mice is about twice as high than in the liver of females, but is only about 15% of that in suckling mice (171). Subcellular distribution studies showed that about 50% of the radioactivity in liver homogenate is localized in the mitochondria; the nuclear and microsomal fractions contain only small amounts of radioactivity. More than 80% of the radioactivity in the mitochondria represents unchanged ^{3}H-luteoskyrin (171). Pretreatment of male mice with 3-methylcholanthrene or promethazine inhibits considerably the accumulation of luteoskyrin in the liver, suggesting that the microsomal mixed-function oxidases play a role in the detoxification of luteoskyrin (123). The pharmacokinetics and the distribution pattern of rugulosin in the mouse was reported to be similar to those of luteoskyrin (cited in rev. 172).

In vitro studies with mitochondrial preparations and whole liver homogenates have shown that luteoskyrin inhibits oxidative phosphorylation through a mechanism similar to that of dinitrophenol in uncoupling phosphorylation and to oligomycin in inhibiting electron transport (173). In the presence of divalent cations (e.g., Mg^{++}, Mn^{++}), luteoskyrin forms complexes with single-stranded as well as double-stranded nucleic acids (174-176). Flow dichroism studies established that luteoskyrin is oriented parallel to the axis of the double helix of native DNA (176). The binding of luteoskyrin to deoxyribonucleohistone in vitro has also been reported (177). Because of its ability

to interact with single-stranded nucleic acids, it was suggested that luteoskyrin binds to nicked DNA and interferes with DNA repair synthesis. Indeed, Mouton and Fromageot (178) showed that the repair of UV-induced DNA lesions in Tetrahymena cells is inhibited by luteoskyrin. There is also evidence that luteoskyrin interacts with the transcription complex and inhibits the synthesis of RNA in Escherichia coli (179).

Rugulosin is believed to have similar DNA-binding properties as luteoskyrin (180).

Cyclochlorotine. Cyclochlorotine is highly resistant to the proteolytic effects of tissue proteases. Following subcutaneous administration to male mice, cyclochlorotine is rapidly absorbed and transported to the liver and is primarily excreted unchanged by the kidney. In vitro studies showed that only specific proteolytic enzymes having an ability to hydrolyze cyclic peptides can degrade cyclochlorotine. Removal of the two chlorine atoms of cyclochlorotine by treatment with ammonia or alkali results in loss of toxicity of the toxin (see rev. 172). Studies with liver preparations have shown that cyclochlorotine inhibits glycogenesis, decreases the incorporation of amino acids into proteins and enhances the incorporation of acetate into lipids (cited in rev. 140). Cyclochlorotine inhibits Na^+-dependent glycine transport in rabbit reticulocytes (158).

5.3.1.2.5 Environmental Significance.

Penicillium toxin-producing fungi can grow at considerably low moisture content and at wide ranges of temperature and pH, and thus occur ubiquitously in the environment. Like the Aspergillus, the Penicillium are among the most common storage fungi in foods throughout the world. Humans may be exposed to Penicillium toxins by direct contact, by inhalation, by therapeutic use or by

ingestion of the contaminated foodstuffs. Although mounting evidence links liver cancer to aflatoxin contamination of food corps (see Section 5.3.1.1), epidemiological evidence on the Penicillium toxin-induced cancer in humans is lacking. This is not too surprising since epidemiological studies on many of these toxins are still in their infancy. Nonetheless, fungal toxins are increasingly suspected to be possible etiological agents of some human cancers (see refs. 181, 182).

Table XVII summarizes the natural occurrence of several carcinogenic Penicillium toxins, which has been the subject of many reviews (e.g., 9, 10, 183).

Ochratoxin A. Ochratoxin A has been detected in corn (0.083-0.166 ppm), wheat (0.03-27 ppm), rye (0.24 ppm), mixed oat and barley (22 ppm), beans (0.02-2.1 ppm) and peanuts (4.9 ppm) during surveys in Canada (184, 185) and in the United States (186, 187). In districts of Denmark where a high incidence of porcine nephropathy occurred, up to 27.5 ppm and 0.067 ppm of ochratoxin A were found in about 20% of the plant (cereals) and animal (pork, poultry) products sampled, respectively. Residues of ochratoxin A have also been detected in various food commodities of seven other European countries (see rev. 188).

Citrinin. Citrinin was detected in 13 of 29 grain samples from Canadian farms at concentrations of 0.07 to 80 ppm. These samples were mainly wheat, but there were also samples of rye, oats and mixed oats, and barley containing citrinin (185). In addition to ochratoxin A, low levels (0.16 to 2 ppm) of citrinin were found in 3 samples of cereals from Denmark (41). There are also reports on the presence of citrinin in moldy ground nut (189) and in rotten apples (190). One of the citrinin-producing fungi (P. citrinum) was isolated from the Japanese "yellowed rice" imported from Thailand (see rev. 17).

Table XVII
Natural Occurrence of Some *Penicillium* Toxins[a]

Toxin	Producing Fungus	Occurrence
Ochratoxin A[b]	*P. viridicatum*; *P. purpurescens*; *P. palitans*; *P. commune*; *P. cyclopium*; *P. variabile*	Corn, wheat, oat, rye, barley, bean, peanut, pork, poultry
Citrinin[c]	*P. citrinum*; *P. citreviride*; *P. viridicatum*; *P. citreo-viride*; *P. fellutanum*; *P. lividum*; etc.	Wheat, oat, barley, groundnut, apple, rice
PR toxin	*P. roqueforti*	Silage
Patulin[d]	*P. patulum*; *P. expansum*; *P. urticae*; *P. cyclopium*; *P. lapidosum*; *P. terrestre*	Apple
Penicillic acid[b]	*P. puberulum*; *P. viridicatum*; *P. thomii*; *P. suavolens*; *P. martensii*; *P. palitans*; *P. expansum*; *P. commune*; *P. olivino-viride*; etc.	Corn, bean, tobacco
Griseofulvin	*P. griseofulvin*; *P. viridicatum*; *P. nigricans*; *P. urticae*; *P. patulum*	Wheat, bean, flour
Penicillin G	*P. chrysogenum*; *P. notatum*	Wheat, flour, rice, fermented foodstuffs
Rubratoxin B	*P. rubrum*; *P. purpurogenum*	Corn, bean, peanut, silage
Luteoskyrin	*P. islandicum*	Rice
Rugulosin[e]	*P. rugulosum*; *P. brunneum*; *P. tardum*; *P. variabile*	Rice
Cyclochlorotine	*P. islandicum*	Rice
Islanditoxin	*P. islandicum*	Rice

[a]References cited in J.M. Bamburg, F.M. Strong and E.B. Smalley, *J. Agr. Food Chem.* 17, 443 (1969); IARC Monographs, Vol. 10, 1976; P.M. Scott, *Penicillium* Mycotoxins, In "Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, An Encyclopedia Handbook" (T.D. Wyllie and L.G. Morehouse, eds.), Vol. 1, Part 2, Marcel Dekker, New York, 1977, p. 283.

[b]Also produced by *Aspergillus ochraceus*, *A. sulphureus*, *A. alliaceus*, *A. sclerotorium*, *A. melleus*, *A. ostianus* and *A. petrakii*.

[c]Also produced by *Aspergillus terreus*, *A. niveus*, *A. candidus* and *Clavariopsis aquatica*.

[d]Also produced by *Aspergillus flavus*, *A. clavatus*, *A. giganteus*, *A. terreus* and *Byssochlamys nivea*.

[e]Also produced by *Myrothecium verrucaria*.

PR Toxin. PR toxin is the major fungal metabolite isolated from moldy silage associated with cases of bovine poisoning in Wisconsin (191). Several strains of PR toxin-producing fungus are used in the ripening of roquefort cheese (see ref. 65).

Patulin. Patulin occurs primarily in rotten apples and related products since patulin-producing fungi are common causes of the storage rot of apples (see rev. 9). The toxin has been detected in 8 of 13 samples of apple juice from the United States at levels of 49 to 309 μg/liter (192). Also, five of 11 apple juice samples from Canada contained 20 to 120 ug patulin/liter (193). The concentration of patulin in apple cider made from rotten apples may be as high as 45 mg/liter (194).

Penicillic acid. Penicillic acid has been identified in moldy corn (195) and in poultry feed (196). Thorpe and Johnson (197) found the toxin in 7 of 20 samples of commercial corn (5-230 μg/kg) and in 5 of 20 samples of commercial dried beans (11-179 μg/kg) from the United States. Snow et al. (198) found 110 and 230 μg/kg of penicillic acid in two samples of moldy tobacco.

Penicillin G. Penicillin was introduced for therapeutic use in the early 1940's. The drug was extracted from cultures of Penicillium notatum. Since then, many new derivatives of the basic penicillin nucleus have been discovered and produced. Presently, members of this important group of antibiotics remain drugs of choice against a wide variety of infectious diseases. Penicillin G is the most effective against infectious diseases caused by gram-positive and gram-negative cocci, gram-positive bacilli, spirochetes, actinomyces and psittacosis virus. Preparations of penicillin G for oral and parenteral administration, as well as for topical, ophthalmic and vaginal uses are all available (see rev. 14).

Penicillin G and several natural penicillins, are presently prepared from a strain of Penicillium chrysogenum that grows on the stem of cantaloupes. High yields of penicillin G are produced by submerged fermentation of a mutant of the mold, induced by x-rays (see rev. 14). Penicillium chrysogenum has been detected occasionally in wheat, rice and in some fermented foodstuffs consumed daily by most Japanese (183, 199).

Griseofulvin. Griseofulvin is produced by many species of Penicillium (see Table XVII). These fungi have been detected in wheat, beans and flour (183). Griseofulvin is often used in human medicine for the treatment of dermatophytoses. The annual sales of griseofulvin in the United States are estimated to be in the order of 25,000 kg (see ref. 10).

Rubratoxin B. Owing to difficulties in detecting rubratoxin B in complex substrates, there are as yet no reports about its natural occurrence in agricultural products. However, fungi that produce rubratoxin B have been repeatedly isolated from cereal and legume products, corn, peanuts and from feeds which have caused liver disease in farm animals (see ref. 168).

Luetoskyrin, Rugulosin, Cyclochlorotine and Islanditoxin. These are commonly referred to as "yellowed rice toxins" because they are metabolites of predominant storage fungi associated with heavily moldy rice ("yellowed rice") of Japan. Contamination by fungi which produce these toxins was found in rice both originated from Japan and imported from Thailand, Burma or other Asian countries. Since rice constitutes a major part of the diet of Asian populations, the high incidence of liver disease, including cancer, has been suspected to be related to consumption of rice contaminated by these carcinogenic toxins (see ref. 200). Yellowed rice toxin-producing fungi are also major isolates from Danish barley as well as from various African grains (see rev. 201).

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