CURRENT AWARENESS DOCUMENT

PYRROLIZIDINE DERIVATIVE ALKYLATING AGENTS AND RELATED PLANT ALKALOIDS:

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

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5.3.2.3 Plant Alkaloids

Plant alkaloids comprise a large group of natural products which are generally basic, nitrogenous heterocyclic compounds designated by the ending "ine." Close to 4,000 of the structurally defined alkaloids are distributed among 8-10% of the flowering plant species (34 out of 60 orders in the higher plant system of Engler) were reported in 1978 (1). Many plant alkaloids are toxic and display a wide spectrum of physiological activities; some of the alkaloids discussed here are also carcinogenic and genotoxic. At one time, colchicine and aristolochic acid were thought to be basic, nitrogenous heterocyclics and were classified as alkaloids. The carcinogenicity of these two compounds is covered in Section 5.3.2.6.4. Arecoline and other betel nut alkaloids are discussed in Section 5.3.2.6.1.

A number of alkaloids also occur in animals, insects, algae, fungi and bacteria (1). Ergot alkaloids, for instance, are present in the fungus, Claviceps purpurea (see Section 5.3.1.4). Information on the toxicology of alkaloids derived from organisms other than plants is still meager.

5.3.2.3.1 Pyrrolizidine Alkaloids

5.3.2.3.1.1 INTRODUCTION

Pyrrolizidine alkaloids constitute a large groups of compounds which occur in plants of a wide botanical and geographical distribution. Close to 200 pyrrolizidine alkaloids have so far been found, distributed among more than 350 plant species belonging to 12 families (principally Compositae, Boraginaceae and Leguminosae) of the angiospermae. Many of these alkaloids are highly hepatotoxic causing acute and chronic illness of grazing livestock and farm animals in many parts of the world. Pyrrolizidine alkaloid poisoning of humans has also occurred through the consumption of contaminated food

grains. In Africa, Asia and other places, some pyrrolizidine alkaloid-containing plants are used as food and folk medicines. Increased interest in pyrrolizidine alkaloids has been stimulated by the findings that a number of these naturally-occurring chemicals, when tested in long-term experiments in animals, are tumorigenic. It is suspected that the high incidence of liver cancer in some populations of the world may be related to the consumption of pyrrolizidine alkaloid-containing plants.

In 1968, Bull, Culvenor and Dick (2) authored a comprehensive monograph on the botanical distribution, chemistry, pathogenicity and other biological properties of pyrrolizidine alkaloids. Subsequently, several periodic reviews, updating information on research in the field, particularly on the toxicology, metabolism and carcinogenic action of the increasing number of pyrrolizidine alkaloids have appeared (e.g., 3-10). Table XLIII lists the names and uses of some plants in which carcinogenic pyrrolizidine alkaloids have been found.

5.3.2.3.1.2 PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL EFFECTS

5.3.2.3.1.2.1 Physical and Chemical Properties. All alkaloids of this class contain in their molecules the pyrrolizidine nucleus bearing a hydroxyl and a hydroxymethyl group; this moiety is termed a necine. Necines form esters with various C_5-C_{10} branched-chain acids, called necic acids. Four types of necines are recognized among the hepatotoxic and carcinogenic pyrrolizidine alkaloids: (a) heliotridine, (b) retronecine, (c) isatinecine (N-oxide of retronecine) and (d) otonecine. The necic acids are saturated or unsaturated, hydroxylated or epoxidized mono- or di-carboxylic acids. Esters formed between necines and necic acids can be classified into: (a) mono-esters, (b) diesters and (c) macrocyclic diesters. The chemical structures of some pyr-

Table XLIII
Names and Uses of Some Plants in Which Carcinogenic Pyrrolizidine Alkaloids Have Been Found[a]

Botanical name	Common name	Human use	Carcinogenic pyrrolizidine alkaloid[b]
Family Compositae:			
Senecio spp.	Ragwort, groundel, stinking Willie, Dan's cabbage, etc.	Medicinal herbs in Africa, Asia, Europe and Jamaica; food in Japan	Retrorsine, isatidine, riddelliine, senkirkine, jacobine, seneciphylline
Tussilago farfara	Coltsfoot	A cough medicine in China, Japan, and Europe; food in Japan	Senkirkine
Petasite japonicus	Coltsfoot	Food or a herbal remedy in Japan	Petasitenine, senkirkine
Farfugium japonicum	"Tsuwabuki" (Japanese)	Food in Japan	Petasitenine, senkirkine
Ligularia dentata			Clivorine
Family Boraginaceae:			
Heliotropium spp.	Common heliotrope, caterpillar weed, potato weed, etc.	Medicinal herbs in India, Greece, and Eastern Mediterranean, Africa and South Africa	Heliotrine, lasiocarpine
Amsinckia intermedia	Fireweed, tarweed, fiddleneck, yellow forget-me-not, etc.		Lycopsamine, intermedine

Table XLIII (continued)

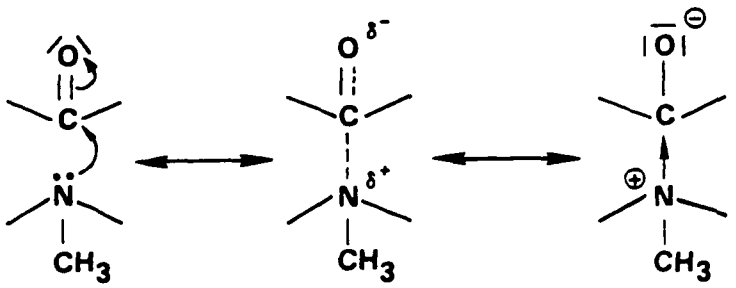
Botanical name	Common name	Human use	Carcinogenic pyrrolizidine alkaloid[b]
Symphytum officinale	Comfrey	Green vegetable and tonic in Japan; medicinal herb in Europe and the United States	Symphytine
Family Leguminosae			
Crotalaria spp.	Rattle box, rattle pad, wild lucerne, earring plant, white back, etc.	Food and medicinal herbs in India and Africa; "bush tea" in West Indies	Retronecine, monocrotaline, retrorsine, isatidine, riddelliine, hydroxysenkirkine, seneciphylline

[a]Summarized from L.B. Bull, C.C.J. Culvenor and A.T. Dick [The Pyrrolizidine Alkaloids. Their Chemistry, Pathogenicity and Other Biological Properties. Wiley, New York, 1968, 293 pp.], E.K. McLean [*Pharmacol. Rev.* 22, 429 (1970)], IARC Monographs, Vol. 10 (1976) and Vol. 31 (1983), and I. Hirono, I. Ueno, S. Aiso, T. Yamaji and M. Haga, *Cancer Lett.*, 20, 191 (1983).

[b]See Table XLIV for structural formulas and Table XLIX for carcinogenicity.

rolizidine alkaloids which have been tested for carcinogenic activity are shown in Table XLIV.

In pyrrolizidine alkaloids containing a ring oxo group (such as in senkirkine, petasitenine and clivorine) the dotted lines represent fractional valence bonds resulting from resonance between the limit structures:



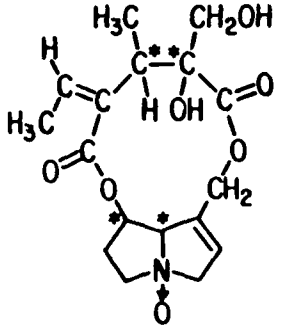
Except for symphytine, which is an oil, the pyrrolizidine alkaloids discussed in this section are crystaline colorless solids, mostly of low to medium melting points. They are all optically active substances. In general, the base strength and solubility in water and organic solvents of pyrrolizidine alkaloids decrease in the order of: non-esters > mono-esters > diesters > macrocyclic diesters. Hydrolysis and dehydrogenolysis of the ester groups are the most important chemical reaction of these alkaloids. Also of importance is the ready interconversion of the pyrrolizidine tertiary bases and their N-oxides which are highly water soluble. Some physicochemical properties of pyrrolizidine alkaloids are summarized in Table XLV.

5.3.2.3.1.2.2 Biological Effects Other Than Carcinogenicity

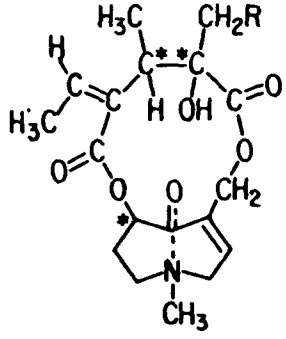
Toxic effects. Pyrrolizidine alkaloid-containing plants have long been recognized as toxic to grazing animals and are responsible for many diseases in farm stock (2). The prominent toxic effects in domestic animals are acute and chronic liver lesions, lung damage, neurological symptoms and hemolytic syndromes (3, 11). In experimental studies with rodents, the most frequently affected organ is the liver. High doses of pyrrolizidine alkaloids cause acute liver necrosis. Small doses produce chronic liver lesions characterized

Table XLIV

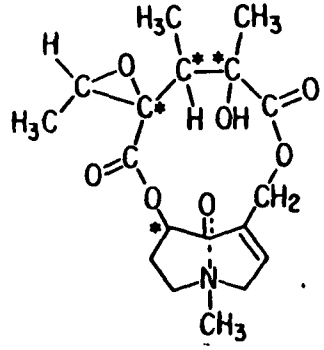
Pyrrolizidine Alkaloids Which Have Been Tested for Carcinogenic Activity



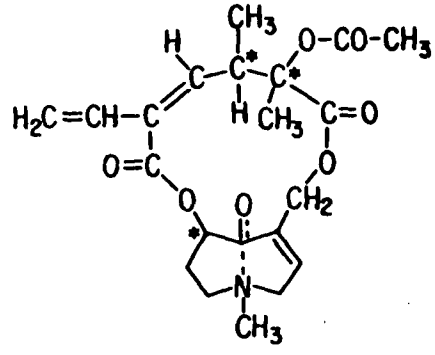
Isatidine



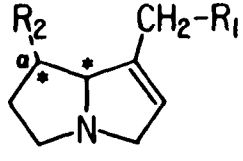
Senkirkine R=-H
Hydroxysenkirkine R=-OH



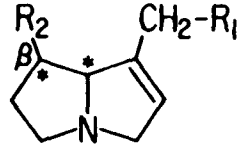
Petasitenine



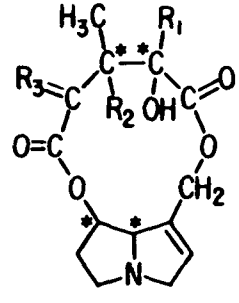
Clivorine



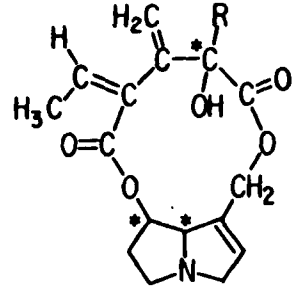
A



B



C

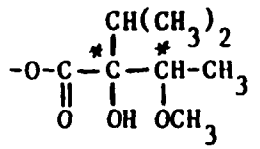
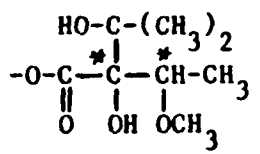
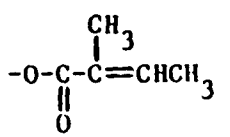


D

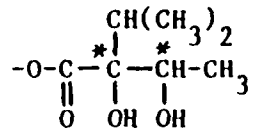
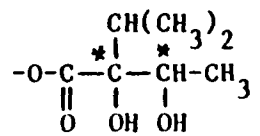
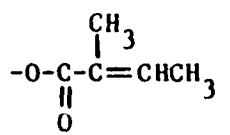
Table XLIV (Continued)

Substituents to Fundamental Ring Structures A, B, C, & D

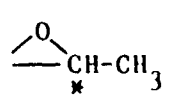
A

	R_1	R_2
Heliotridine	-OH	-OH
Heliotrine		-OH
Lasiocarpine		

B

	R_1	R_2
Retronecine	-OH	-OH
Lysopsamine		-OH
Symphytine		

C

	R_1	R_2	R_3
Retrorsine	$-CH_2OH$	-H	$=CH-CH_3$
Monocrotaline	$-CH_3$	-H	$\begin{cases} -CH_3 \\ -H \end{cases}$
Jacobine	$-CH_3$	-H	

D

Seneciphylline	$R = -CH_3$
Riddelline	$R = -CH_2OH$

*asymmetric carbon

Table XLV
Physicochemical Properties of Some Carcinogenic Pyrrolizidine Alkaloids[a]

Compound[b]	M.p. (°C)	$[\alpha]_D$ (solvent)	pKa		Solubility
			In 80% MCS[d]	In water	
Heliotrine[c]	128	+17.6° (EtOH)	7.82	8.52	2.64 g/dl; soluble in ethanol
Lasiocarpine[c]	96.5-97	-3.0° (EtOH)	6.55	7.64	0.68 g/dl; soluble in ethanol and most non-polar solvents
Retronecine	117-118	+49.6° (EtOH)	8.38		Soluble in ethanol
Lycopsamine		+3.3° (EtOH)		8.5	Soluble in water and ethanol
Intermedine	146.5-147	+4.7° (EtOH)		8.5	Soluble in water and ethanol
Symphytine		+3.65° (EtOH)			Soluble in ethanol
Retrorsine	216-216.5	-18.0° (EtOH) -62.0° ($CHCl_3$)			Soluble in chloroform; slightly soluble in water and ethanol
Monocrotaline[c]	202-203	-15.0° (EtOH) -55.0° ($CHCl_3$)			1.21 g/dl; soluble in ethanol and chloroform
Jacobine	228	-46.3° ($CHCl_3$)	6.04		Soluble in chloroform; sparingly soluble in water, ethanol and ether
Riddelliine	198	-109.5° ($CHCl_3$)	6.30		Soluble in chloroform; slightly soluble in water and ethanol
Seneciphylline	217	-139.0° ($CHCl_3$)	6.20	7.6	Soluble in chloroform; slightly soluble in water and ethanol

Table XLV (continued)

Compound[b]	M.p. (°C)	$[\alpha]_D$ (solvent)	pKa In 80% MCS[d]	pKa In water	Solubility
Isatidine	138	-8.2° (H_2O)			Soluble in water and ethanol
Senkirkine	197-198	-12.0° (EtOH) -2.0° ($CHCl_3$)			Soluble in chloroform and ethyl acetate; less soluble in water and ethanol
Hydroxysenkirkine	124-125	$+5.3^\circ$ (EtOH)			Soluble in water, ethanol, chloroform and hot acetone
Petasitenine	129-131	$+63.8^\circ$ ($CHCl_3$)			Highly soluble in water
Clivorine	149-150	$+80.2^\circ$ ($CHCl_3$)			Soluble in chloroform

[a]Summarized from L.B. Bull, C.C.J. Culvenor and A.T. Dick, "The Pyrrolizidine Alkaloids. Their Chemistry, Pathogenicity and Other Biological properties." Wiley, New York, 1968, 293 pp.]; IARC Monograph Vol. 10, International Agency for Research on Cancer, Lyon, France, 1976; T. Furuya, M. Hikichi and Y. Iitaka [Chem. Pharm. Bull. 24, 1120 (1976)]; and K. Kuhara, H. Takanashi, I. Hirono, T. Furuya and Y. Asada [Cancer Lett. 10, 117 (1980)].

[b]See Table XLIV for structural formulas.

[c]Half-lives of alkaloids (in 0.5N NaOH at 25°C) in 1:1 aqueous ethanol: heliotrine = 8 days, lasiocarpine = 20 min., and monocrotaline = 18 min.; partition coefficients of alkaloids in oleyl alcohol/pH 7.3 buffer: heliotrine = 0.11, lasiocarpine = 2.5, and monocrotaline = 0.082.

[d]MSC = methyl cellosolve.

by the appearance of large parenchymal cells, "megalocytes." Some alkaloids, especially mono-crotaline and seneciphylline, also cause lesions of the lung (12). Liver necrosis and hepatic "veno-occlusive disease" are frequently seen in humans ingesting pyrrolizidine alkaloid-containing plants as contaminants of grain or as herbs for medicinal purposes (3, 13-16).

There is an inverse relationship between the acute hepatotoxicity and both the water solubility and base strength of the pyrrolizidine alkaloids (17). In order to be toxic, the compounds must contain the 1-hydroxymethyl-pyrrolizidine structure, be unsaturated at the 1,2-position, and esterified at one of the hydroxy groups. In general, macrocyclic diesters of retronecine, such as retrorsine, are the most toxic alkaloids. Among the open esters, diesters are more toxic than the monoesters; those of heliotridine are more so than those of retronecine (12, 18). As compared with the corresponding alkaloids, pyrrolizidine N-oxides are less acutely toxic when administered parenterally but are similar in chronic effects (18). The acute toxicities (LD_{50} values) of some pyrrolizidine alkaloids in rats and mice are given in Table XLVI.

Considerable evidence supports the conclusion that the hepatotoxic effects of pyrrolizidine alkaloids are attributable to the pyrrolic metabolites formed in the liver by enzymatic dehydrogenation (see Section 5.3.2.3.1.4). The enzyme system(s) involved has many characteristics of mixed-function oxidases (20, 25). Factors such as species, age, sex, diet and drug pretreatment, all of which influence the activity of mixed-function oxidases, alter the hepatotoxicity of pyrrolizidine alkaloids in animals (e.g., 26-28). Among livestock species, for instance, cattle and horses are more susceptible than sheep and goat to pyrrolizidine alkaloid toxicity (2, 29). In laboratory animals, rats, mice and hamsters are more susceptible than

Table XLVI
Acute Toxicity of Some Pyrrolizidine Alkaloids

Compound[a]	Species and route	LD_{50} (mg/kg)[b]	Reference
Retrorsine	Rat, i.p.	35 (M); 153 (F)	(18, 19)
	i.v.	38	(2)
	Mouse, i.v.	59	(2)
	i.p.	65 (M); 69 (F)	(2)
	Hamster, i.p.	81	(20)
Lasiocarpine	Rat, i.p.	72 (M), 79 (F)	(2)
	i.v.	88	(2)
	Mouse, i.v.	85	(2)
	Hamster, i.v.	68	(2)
Monocrotaline	Rat, i.p.	95 (M), 180 (F)	(19)
	i.v.	92	(2)
	Mouse, oral	166	(2)
	i.v.	261	(2)
Isatidine	Rat, oral	48	(21)
	i.p.	250	(19, 21)
	Mouse, i.v.	835	(22)
Heliotrine	Rat, i.p.	300 (M)	(2)
	i.v.	274	(2)
	Mouse, i.v.	254	(2)
Senkirkine	Rat, i.p.	220	(23)
Seneciphylline	Rat, i.p.	77 (M), 83 (F)	(2)
	i.v.	80	(2)
	Mouse, i.v.	90	(2)
Jacobine	Rat, i.p.	138 (F)	(2)
	Mouse, i.v.	77	(2)
Riddelliine	Mouse, i.v.	105	(2)
Lycopsamine + intermediate	Rat, i.p.	>1,000 (M)	(2)
Symphytine	Mouse, i.p.	300	(24)
	Rat, i.p.	130	(23)

[a]See Table XLIV for structural formulas.

[b]Abbreviation: M = male, F = female.

rabbits and guinea pigs (20, 30). In general, the males are more sensitive than females and the newborns are more susceptible than older animals (26). The protective effect of zinc on pyrrolizidine alkaloid-induced hepatotoxicity in rats is primarily due to the blockage by the metal of the microsomal conversion of the parent compounds to their toxic metabolites (28).

Acting either as mono- or bi-functional alkylating agents, the pyrrolic metabolites of many pyrrolizidine alkaloids bind to, and inhibit, the synthesis of cellular macromolecules (e.g., 17, 31-33). These metabolites are also known to arrest mitosis of liver cells resulting in the development of the chronic liver lesions, megalocytosis (31, 34).

Mutagenic effects. The mutagenic and genotoxic activities of some carcinogenic pyrrolizidine alkaloids have been demonstrated in several assay systems. The results of these studies are summarized in Table XLVII.

In microbial systems, preincubation of those alkaloids with S-9 mix is required for the appearance of mutagenic activity. Yamanaka and coworkers (35) noted that several pyrrolizidine alkaloids of the heliotridine (e.g., heliotrine and lasiocarpine) and otonecine (e.g., clivorine, petasitenine and senkirkine) base types give positive mutagenic response in the Salmonella/mammalian microsomal test. On the other hand, pyrrolizidine alkaloids of retronecine base type (e.g., lycopsamine, monocrotaline, retronecine and seneciphylline) were not mutagenic under the same study conditions. These observations led Yamanaka and associates to suggest that pyrrolizidine alkaloid mutagenicity might be related to the necine base type of the compounds. This view appears to be supported by findings of other investigators that lasiocarpine (36) but not monocrotaline and jacobine (38), exhibit mutagenic response in Ames strains of Salmonella typhimurium. Moreover,

Table XLVII
Mutagenicity and Related Genotoxic Activities of Some Pyrrolizidine Alkaloids

Compound[a]	Salmonella typhimurium[b]	Escherichia coli	Aspergillus nidulans	Drosophila melanogaster[c]	V79 Chinese hamster cell[d]	DNA repair	Chromosomal aberrations
Heliotrine (H)	+ (35)	+ (39)	+ (40)	+ (41)	+ (44)		+ (44)
Lasiocarpine (H)	+ (35, 36)		+ (40)	+ (41)	+ (44)	+ (45)	+ (44)
Senkirkine (O)	+ (35)			+ (42)	+ (44)	+ (45)	+ (44)
Petasitenine (O)	+ (35)				+ (44)	+ (45)	+ (44)
Clivorine (O)	+ (35)					+ (45)	
Retrorsine (R)	+ (37)			+ (43)		+ (46)	
Symphytine (R)	+ (9)				+ (44)		
Monocrotaline (R)	- (35, 38)	+ (39)		+ (41)		+ (45)	+ (47)
Jacobine (R)	- (38)			+ (41)			
Seneciphylline (R)	- (35)			+ (42)			
Retronecine (R)	- (35)						
Lycopsamine (R)	- (35)						
Isatidine (I)				+ (43)			

[a]Letters in parentheses are necine base types: H = heliotridine; O = otonecine; R = retronecine; I = isatinecine; see Table XLIV for structural formulas.

[b]In the presence of S-9 mix; "+" = positive; "-" = negative; numbers in parentheses are references.

[c]Relative mutagenic activity: lasiocarpine = 1.0; heliotrine = 0.9; monocrotaline = 1.6; jacobine = 0.08.

[d]8-Azaguanine-resistant mutation assay.

retrorsine (37) and symphytine (cited in ref. 10), both of retronecine base type, induce mutation in these bacterial strains in the presence of S-9 mix. The genotoxic DNA-damaging activity of monocrotaline and heliotrine could, however, be shown in some repair-deficient strains of Escherichia coli which proved to be more sensitive than Salmonella (39). Heliotrine and lasiocarpine are also mutagenic in the fungus, Aspergillus nidulans (40).

Early studies with Drosophila melanogaster have indicated that monocrotaline, lasiocarpine and heliotrine are potent mutagens; their N-oxides are less active. Jacobine is only weakly mutagenic. The relative potencies of these alkaloids with respect to mutagenicity in Drosophila are: lasiocarpine = 1.0; heliotrine = 0.9; monocrotaline = 1.6; jacobine = 0.08 (41). More recently, retrorsine, isatidine, senkirkine and seneciphylline have also been shown to induce sex-linked recessive lethals in Drosophila (42, 43).

When tested in V79 cells derived from Chinese hamster lung, heliotrine, lasiocarpine, petasitenine and senkirkine all induced chromosomal aberrations and an 8-azaguanine-resistant mutation (44). Clastogenic activity was also observed in Chinese hamster ovary cells following exposure to monocrotaline in the presence of a microsomal activation system (47).

Williams et al. (45) have demonstrated the genotoxicity of lasiocarpine, petasitenine, senkirkine, clivorine and monocrotaline in the hepatocyte primary culture/DNA repair test. Retrorsine induces DNA repair replication in livers of the rat (46).

A number of pyrrolizidine alkaloid-containing plants have also been evaluated for the presence of mutagenic substances. With the addition of liver microsomes from various mammalian species, an acetone extract of tansy ragwort (Senecio jacobaea) produced positive mutagenic response in the tester

strains of TA1535, TA1537, TA98 and TA100 of Salmonella typhimurium (38). A methanol extract of coltsfoot (Petasites japonicus) was also shown to be mutagenic in the Salmonella strain, his G46, in an in vivo host-mediated assay (48). Extracts of fresh leaves of the Russian comfrey, Symphyton officinale, are mutagenic in the sex-linked recessive lethal test in Drosophila (49). The mutagenic substance(s) in extracts of these plants remains to be investigated.

Teratogenic effects. Experimental data on teratogenic studies of pyrrolizidine alkaloids are surprisingly scanty. In the light of the alkylating potential and antimitotic activity of their metabolites (see Section 5.3.2.3.1.4), it is likely that pyrrolizidine alkaloids might be teratogenic. Green and Christie (50) have demonstrated a positive teratogenic effect of heliotrine in the rat. Various skeletal malformations were observed in offspring of rats receiving single doses (100 mg/kg) of heliotrine intraperitoneally during the second week of gestation. Other investigators have shown that the administration of lasiocarpine to pregnant (51) or to lactating (52) rats, in doses non-toxic to the dams, caused significant liver lesions in the newborns and weanlings.

5.3.2.3.1.3 CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS

Overview. Pyrrolizidine alkaloids are among the first naturally occurring carcinogens found in products of plant origin. A report of tumor induction by pyrrolizidine alkaloids dates back to 1950 when Cook, Duffy and Schoental (53) described the development of hepatomas in rats following feeding with an alkaloidal fraction from tansy ragwort (Senecio jacobaea). Since then, several other plants or plant extracts containing pyrrolizidine alkaloids were shown to produce neoplasms in laboratory animals (rev. in 8, 54). The results of these studies are presented in Table XLVIII. While most

Table XLVIII
Carcinogenicity of Some Pyrrolizidine Alkaloid-Containing Plants

Plant	Part[a]	Carcinogenicity	Major pyrrolizidine alkaloids	Reference
Senecio jacobaea	Stem and leaf or plant extract	Liver tumors in rats and chicks	Seneciphylline[b], jacobine[b], senecionine, jaconine, jacoline, jacodine	(53, 55, 56)
S. aquatics		Liver tumors in rats	Seneciphylline[b]	(57)
S. longilobus	Stem and leaf	Liver tumors in rats	Seneciphylline[b], retrorsine[b], riddelliine[b]	(58)
S. cannabifolius	Stem and leaf	Liver tumor in rats	Seneciphylline[b], senecicannabine, jacozine	(59)
Farfugium japonicum	Stem and leaf	Liver tumors in rats	Senkirkine[b], petasitenine[b]	(59)
Heliotropium supinum	Stem and leaf or plant extract	Pancreas and kidney tumors in rats	Supinine, echinatine, heliosupine, trachelanthyl-7-angelylheliotridine, viridofloryl-7-angelylheliotridine	(60, 61)
H. ramosissimum	Stem and leaf	Brain tumors in rats	Heliotrine[b]	(62)
Amsinckia intermedia	Seeds	Kidney tumors in rats	Lycopsamine[b], intermedine[b]	(61)
Petasites japonicus	Flower stalk	Liver tumors in rats and mice	Petasitenine[b]	(54, 63-65)
Tussilago farfara	Flower	Liver tumors in rats	Senkirkine[b], senecionine	(54, 66)
Symphytum offinale	Root and leaf	Liver tumors in rats	Symphytine[b], echimidine	(54, 66)

[a]Plant parts were mixed and fed with the diet; plant extracts were injected intravenously.

[b]Carcinogenic activity has been tested; see Table XLIX.

of these plants (or their extracts) induce tumors in the liver of rats, dried plants (stems and leaves) of the genus Heliotropium elicit neoplasms of the pancreas, the kidney (H. supinum) and the brain (H. ramosissimum) in the rat (60-62). Heliotropium supinum and H. ramosissimum are medicinal herbs used in East Africa (60, 62). Senecio cannabifolius, Petasites japonicus, Tussilago farfara, Farfugium japonicum and Symphytum officinale are regarded as edible plants in Japan (54, 59).

To date, only a few alkaloidal constituents of these plants have been studied in long-term experiments for carcinogenic activity. However, all hepatotoxic pyrrolizidine alkaloids and their metabolites, tested to date under adequate conditions have been found to be carcinogenic in the rat, inducing tumors not only in the liver, but also in various other organs. The carcinogenic pyrrolizidine alkaloids encompasses members of the monoester, diester and macrocyclic diester categories (see Table XLIV for structural formulas). The macrocyclic diester alkaloids appear to be more potent carcinogens than the monocyclic, open esters. Retronecine, which is not hepatotoxic, is also carcinogenic when given to newborn rats. The carcinogenicity studies of these compounds are summarized in Table XLIX. Retrorsine, monocrotaline, dehydroretronecine (85) and petasitenine (54) also exhibit positive effects in in vitro cell transformation assays. Structure-activity relationship analysis suggest that the double bond in the necine ring (1,2-dehydropyrrolizidine) is essential for transforming cells in vitro (85).

Heliotrine. The possible carcinogenic effect of heliotrine, which occurs in Heliotropium ramosissimum and several other species (2, 86), has been investigated by Schoental (67) in male weanling Porton-Wistar rats. All animals given one or two doses of heliotrine (300 mg/kg body weight) by stomach tube died within 5 months. When the dose of heliotrine was reduced to

Table XLIX
Carcinogenicity of Pyrrolizidine Alkaloids

Compound[a]	Species and strain	Route	Principal organ affected	Reference
Heliotrine	Rat, Porton-Wistar	oral	Pancreas, liver, urinary bladder and testis	(67)
Lasiocarpine	Rat, Fischer	i.p.	Liver, skin	(6, 68, 69)
	Rat, Fischer 344	oral	Liver, hemato-poietic tissue	(70, 71)
	Rat, Fischer 344	oral	Liver, hemato-poietic tissue	(72)
Dehydroheliotridine	Rat, hooded	i.p.	Multiple sites	(73)
Retronecine	Rat, Wistar	s.c.	CNS	(62)
Lycopsamine, intermedine (mixture)	Rat, Porton-Wistar	oral	Pancreas	(60)
Symphytine	Rat, ACI	i.p.	Liver	(23)
Retrorsine	Rat, Wistar	oral or i.p.	Liver	(55, 74, 75)
	Rat, Wistar	oral	Kidney	(61)
Isatidine	Rat, Wistar	oral, i.p. or topical	Liver	(55, 74)
Monocrotaline	Rat, Wistar	oral, i.p. or topical	Liver, lung	(76)
	Rat, Sprague-Dawley	oral	Liver	(77)
	Rat, Sprague-Dawley	s.c.	Multiple sites	(78, 79)
	Rat, Sprague-Dawley	s.c.	Pancreas	(80)
Dehydromonocrotaline	Mouse, LACA	topical	Skin	(81)

Table XLIX (continued)

Compound[a]	Species and strain	Route	Principal organ affected	Reference
Jacobine, seneciphylline (mixture)	Chick, --	i.v.	Liver	(56)
Riddelliine	Rat, Wistar	oral and i.p.	Liver	(74)
Dehydroretronecine	Rat, Sprague-Dawley	s.c.	Muscle	(78, 79)
	Mouse, Swiss	s.c. and/or topical	Skin	(82)
	Mouse, LACA	topical	Skin	(81)
Senkirkine	Rat, ACI	i.p.	Liver	(23)
Hydroxysenkirkine	Rat, Wistar	i.p.	CNS	(62)
Petasitenine (Fukinotoxin)	Rat, ACI	oral	Liver	(83)
Clivorine	Rat, ACI	oral	Liver	(84)

[a]See Table XLIV for structural formulas.

230 mg/kg, one rat survived 27 months and developed adenomas of the pancreatic islet cells. Co-administration of nicotinamide was proved to prevent liver necrosis and suppress the toxicity of heliotrine. Six of 12 rats receiving one or two intragastric doses of heliotrine (230 mg/kg) together with nicotinamide (350 mg/kg) by intraperitoneal injections, survived 22 months or longer. Among the survivors, 3 had pancreatic islet cell tumors, accompanied in one of them by a hepatoma and in another by tumors of the urinary bladder and testis. Such tumors were not found in control rats.

Lasiocarpine. The carcinogenicity of lasiocarpine has been repeatedly demonstrated in Fischer rats by Reddy and his coworkers (68-71). In one experiment, lasiocarpine was administered intraperitoneally to 25 male rats at doses of 7.8 mg/kg body weight, twice weekly for 4 weeks and then once weekly for 52 weeks. Of the animals surviving after the treatment period, 61% (11/18) developed hepatocellular carcinomas, 33% (6/18) developed squamous-cell carcinomas of the skin, and 28% (5/18) had pulmonary adenoma (68, 69). Both the carcinomas of the liver (68) and of the skin (69) were transplantable. At doses of 0.39, 0.78 and 1.56 mg/kg, lasiocarpine also induced low incidences of liver neoplasms in male and female rats by repeated i.p. injections (6). When lasiocarpine was fed to 20 male rats in the diet (50 ppm) for 55 weeks, malignant tumors were found in 17 animals between 48 and 59 weeks: 9 developed angiosarcomas of the liver (1 of these also had carcinomas of the skin), 7 developed hepatocellular carcinomas, and one developed lymphomas. The angiosarcoma from one rat was successfully transplanted through 4 generations (70). Administration of thioacetamide, a liver carcinogen which stimulates cell proliferation (see section 5.2.2.6, Vol. IIIB) stimulates the development of hyperplastic nodules and carcinomas of the liver of rats treated with lasiocarpine (71, 87).

Under the bioassay conditions of the U.S. National Cancer Institute (72), lasiocarpine is also carcinogenic in Fischer 344 rats, producing angiosarcomas and hepatocellular tumors in both sexes and hematopoietic tumors in female animals. Statistically significant incidences of these neoplasms were observed when groups of 24 rats of each sex were administered lasiocarpine in the diet at doses of 7, 15 or 30 ppm for 104 weeks.

Dehydroheliotridine. Dehydroheliotridine is a major metabolite of heliotridine-base alkaloids such as heliotrine and lasiocarpine (see Section 5.3.2.3.1.4 on Metabolism and Mechanism of Action). When a group of 24 hooded strain rats was injected with dehydroheliotridine intraperitoneally once every 4 weeks for 32 weeks (first dose, 76.5 mg/kg; second dose, 65 mg/kg; remainder of doses, 60 mg/kg), 11 malignant and benign tumors emerged in 6 animals. In addition to one cystic cholangioma in the liver, other neoplasms occured in the pancreas, lung, adrenal gland, forebrain and the gasotrintestinal tract. This wide spectrum of tumors indicates that the tissue targets of dehydroheliotridine are predominantly extrahepatic. Co-administration of thioacetamide enhances the hepatic toxicity but not the carcinogenicity of dehydroheliotridine (73).

Retronecine. Retronecine is the only non-hepatotoxic and non-esterified pyrrolizidine alkaloid that is carcinogenic. In a small-scale study, a spinal cord tumor was noted in one of 10 male Wistar rats 201 days after a single s.c. dose (600 mg/kg) of retronecine, administered when the rats were newborn. Such tumors were not found in hundreds of historical control rats. Among 6 female newborn rats given a single s.c. injection of 1,000 mg/kg retronecine, 5 developed pituitary tumors and one had a mammary tumor (62).

Lycopsamine and Intermedine. These two alkaloids are stereoisomers which may be isolated from tarweed (Amsinckia intermedia), a plant known to cause severe livestock losses from liver injuries in the United States. One adenoma and one adenocarcinoma of the islet cells and one adenoma of the exocrine pancreas were observed in 3 of 15 male Wistar rats given single doses (500-1,500 mg/kg) of a mixture of lycopsamine and intermedine by stomach tube. Pancreatic tumors of these types were considered to be very rare in these animals (60).

Symphytine. The carcinogenicity of Russian comfrey (Symphytum officinale) is attributed to symphytine. Among 20 male ACI rats which received i.p. injections of symphytine at the dose of 13 mg/kg body weight (10% of the LD_{50}), twice weekly for 4 weeks and then once a week for 52 weeks, one had a liver cell adenoma and 3 developed hemangioendothelial sarcomas in the liver. These tumors were similar to the ones observed in rats fed diets containing roots and leaves of Russian comfrey (88).

Retrorsine. The chronic liver lesions produced in rats by this highly toxic alkaloid were noted by Schoental et al. (55) in early 1954. Fourteen Wistar rats (10 male and 4 female) which were administered 0.03-0.05 mg/ml retrorsine in the drinking water 3 days a week, survived from 10 to 24 months. On examination, nodular or microscopic foci of hyperplasia of the liver were found in 9 of the 10 male rats. The nodules in 4 of these rats were histologically identified as hepatomas. Subsequent studies, in which weanling Wistar rats were given repeated i.p. doses (74) or single large doses (30 mg/kg) of retrorsine by stomach tube (75), also showed significant incidences of tumors. In addition to hepatomas, other neoplasms observed in the retrorsine-treated rats were tumors of the lung (55, 75), mammary gland, spleen, uterus (75) and kidney (61).

Isatidine (Retrorsine-N-oxide). The N-oxide of retrorsine seems to be even more hepatocarcinogenic than its parent compound. Of 29 Wistar rats of both sexes receiving 0.03-0.05 mg/ml of isatidine in the drinking water, 13 developed neoplasms and hyperplastic nodules of the liver; the tumors in one of the rats metastasized. Although no tumors were found in the liver, 8 other rats treated with isatidine had preneoplastic nodules. Supplements with choline did not protect the liver from the carcinogenic action of isatidine (55). Liver tumors are also induced in the rat when isatidine is administered by painting on the neck (55) or by i.p. injection (74).

Monocrotaline. Although monocrotaline has long been implicated as a carcinogen, conclusive data have not been reported until recently. In 1955, Schoental and Head (76) noted lesions of the liver and lung indicative of neoplasia in Wistar rats exposed chronically to monocrotaline. Newberne and Rogers (77) reported later that hepatocellular carcinomas were produced in 31% (24/77) of Sprague-Dawley rats receiving repeated doses (25 mg/kg for 4 weeks then 8 mg/kg for 38 weeks) of the alkaloid by stomach tube. Allen and coworkers (78, 79) administered monocrotaline (5 mg/kg body weight) biweekly, by s.c. injection to a group of 60 male Sprague-Dawley rats for a year. Twelve months after the treatment, 10 animals had pulmonary adenocarcinomas, 5 had well-differentiated hepatocellular carcinomas and 4 developed rhabdomyosarcomas. Additionally, 8 adrenal adenomas, 3 acute myelogenous leukemias and one renal adenoma were observed in the treated rats. A high incidence (70%) of insuloma of the pancreas also occurred in 23 male Sprague-Dawley rats 500 days after a single subcutaneous injection of 40 mg/kg monocrotaline (80).

Dehydromonocrotaline. The carcinogenic potential of dehydromonocrotaline, a putative primary metabolite of monocrotaline (see Section 5.3.2.3.1.4 on Metabolism and Mechanism of Action) has been assayed by mouse skin-

painting. Repeated topical doses (2.5 μmol) of dehydromonocrotaline, followed by repeated treatment with croton oil, resulted in the development of malignant tumors of the skin in 5 of 10 LACA strain mice (81). Without the tumorigenesis promotor, no carcinogenic effect was observed (81, 89).

Jacobine and Seneciphylline. A mixture of jacobine and seneciphylline (primarily seneciphylline), purified from ragwort (Senecio jacobaea), was tested for carcinogenic activity in the chick (56). Primary liver tumors occurred in 6 of 24 chicks receiving weekly i.v. doses (20-35 mg/kg) of the alkaloid mixture for up to 8 weeks or until death.

The chronic changes in the liver of rats caused by seneciphylline are closely similar to those in rats given lasiocarpine or other carcinogenic pyrrolizidine alkaloids. Liver hyperplastic nodules developed in Wistar rats after a single oral dose (40 mg/kg) of seneciphylline (90). Plants containing seneciphylline and jacobine have been shown to produce liver tumors in the rat (see Table XLVII).

Riddelliine. Riddelliine occurs in the carcinogenic plant Senecio longilobus, as well as in several other Senecio species (2). Chronic administration of this alkaloid to rats produced liver lesions characteristic of many hepatocarcinogenic alkaloids of this class. In a chronic study in which 20 male and female Wistar rats were given riddelliine in their drinking water (0.02 mg/kg) and by i.p. injections (25 mg/kg), 9 animals had hyperplastic nodules of the liver and one had a hepatic sarcoma. No such lesions were found in 15 controls (74).

Dehydroretronecine. Dehydroretronecine is the secondary metabolite of monocrotaline and perhaps of other retronecine-based alkaloids (see Section 5.3.2.3.1.4 on Metabolism and Mechanism of Action). The direct-acting carcinogenic effects of this metabolite have been studied in both rats and mice.

Of 60 male Sprague-Dawley rats receiving biweekly s.c. injections of dehydroretronecine (20 mg/kg for 4 months, then 10 mg/kg for 8 months), 39 developed rhabdomyosarcomas at the injection site. Metastasis occurred in 5 cases (78, 79). Significant incidences of basal cell and squamous cell carcinomas of the skin were found in 92 Swiss mice given repeated s.c. injections and/or topical applications of dehydroretronecine (82). Repeated topical doses (5 μmol/dose) of dehydroretronecine also induced skin tumors, in mice of the BALB/c and LACA strains (81, 89). Similar carcinogenicity toward the skin of LACA strain mice were shown by the synthetic compounds, 2,3-bistrimethylacetoxymethyl-1-methylpyrrole and 2,3-bis-hydroxymethyl-1-methylpyrrole but not by 2,3-bis-hdyroxymethyl-5-methyl-1-phenylpyrrole (see Table L).

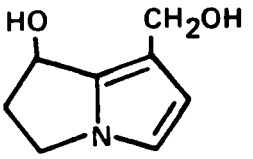
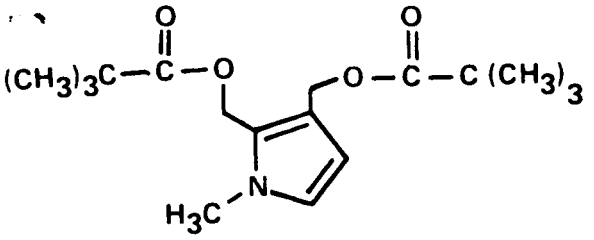
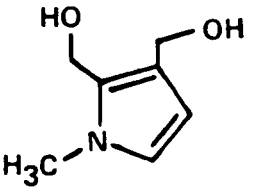
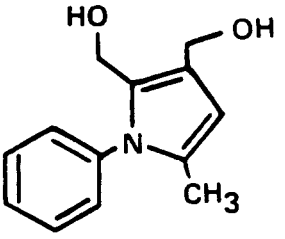
Senkirkine. The active compound in coltsfoot (Tussilago farfara) is senkirkine. When a group of 20 ACI strain rats were given i.p. injections of senkirkine (22 mg/kg) freshly prepared from the flowers of the plant, twice weekly for 4 weeks and then once a week for 52 weeks, 9 animals developed liver cell adenomas similar to those observed in rats fed diets containing the flowers of coltsfoot (23).

Hydroxysenkirkine. Schoental and Scavanagh (62) reported that a single i.p. dose of hydroxysenkirkine (300 mg/kg), isolated from an East African plant Crotalaria laburnifolia (a variety of laburnifolia), induced an astrocytoma of the cerebrum in one of 4 male rats of the Wistar-Porton strain. Tumors of this type were not seen among hundreds of historical controls.

Petasitenine (Fukinotoxin). There is evidence indicating that petasitenine may be responsible for the carcinogenic activity of Petasites japonicus, a variety of coltsfoot (83). The same types of tumors (i.e., hemangioendothelial sarcomas and liver cell adenomas) were found in 8/10 ACI

Table L

Relative Carcinogenic Activity of Dehydroretronecine and Structurally-Related Compounds Toward the Skin of LACA Mice[a]

Compound	Structure	Dose (μmol/mouse)[b]	Skin tumo incidence
Dehydroretronecine		5.0	25%
2,3-Bis-trimethylacetoxy-methyl-1-methylpyrrole		0.5	90%
2,3-Bis-hydroxymethyl-1-methylpyrrole		5.0	25%
2,3-Bis-hydroxymethyl-5-methyl-1-phenylpyrrole		5.0	9%[c]

[a]Modified from: A.R. Mattocks and J.R.P. Cabral [Cancer Lett. 17, 61 (1982)].

[b]Applied to the shaved backs of mice at weekly intervals for up to 47 weeks.

[c]Statistically not significant as compared with control incidence.

strain rats receiving a 0.01% solution of petasitenine in the drinking water, as in the rats fed a diet containing the flower stalks of Petasites japonicus (54, 63, 64).

Clivorine. Like senkirkine, hydroxysenkirkine and petasitenine, clivorine is a pyrrolizidine alkaloid of the macrocyclic diester type containing otonecine as the necine base. Among 12 ACI strain rats ingesting a 0.005% solution of clivorine in drinking water for 340 days, 2 developed hemangioendothelial sarcomas and 6 had neoplastic nodules of the liver. The hemangioendothelial sarcoma in one rat showed metastasis in the lung. No liver tumors or nodules were found in the controls (84).

5.3.2.3.1.4 METABOLISM AND MECHANISM OF ACTION

There is considerable evidence that the toxicological effects of pyrrolizidine alkaloids are not due to the compounds themselves but to metabolites formed in the liver. Much of the metabolic studies of these toxic alkaloids have been carried out in the laboratory of Mattocks, White and associates in Great Britain (20, 91-94). The general scheme for the metabolism of some carcinogenic pyrrolizidine alkaloids is shown in Fig. 11.

Pyrrolizidine alkaloids which are esters of heliotridine (e.g., heliotrine, lasiocarpine) or retronecine (e.g., lycopsamine, symphytine, retrorsine, monocrotaline, jacobine, riddelliine and seneciphylline) undergo N-oxidation to yield N-oxide derivatives, and C-hydroxylation to give dehydroalkaloids (primary pyrrolic derivatives) in the liver of the rat. In vitro studies have shown that both reactions are catalysed by typical mixed-function oxidases in the microsomes (91). The formation of N-oxides, which are more water soluble and have low toxicity, is considered to be a detoxifying reaction. The toxic effects observed following administration of N-oxide

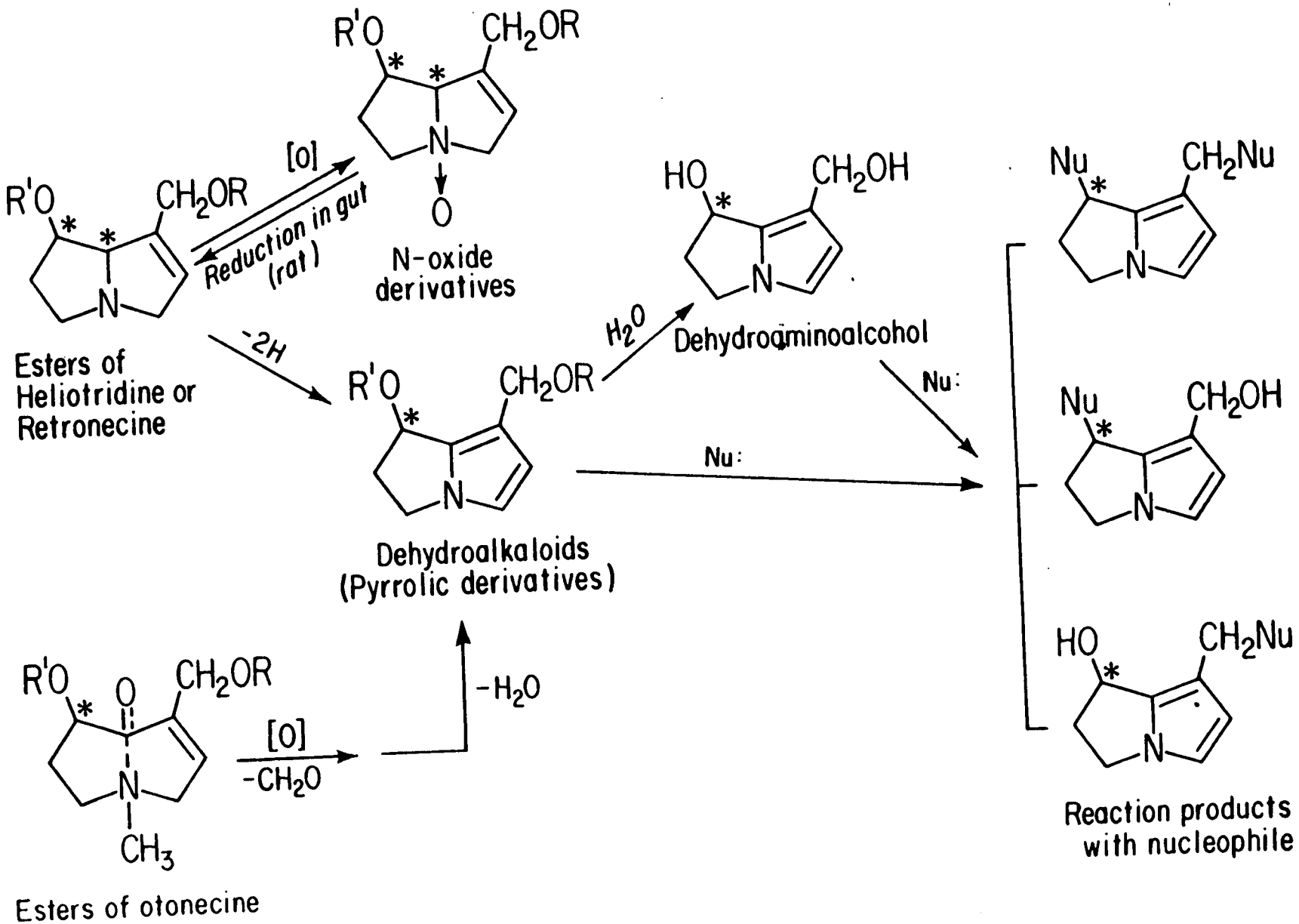
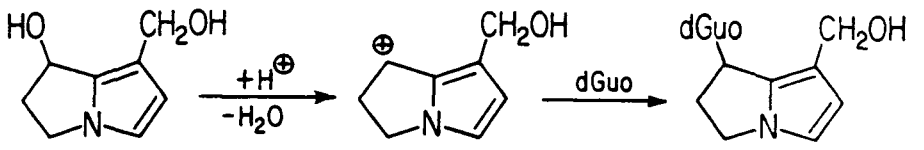


Fig. 11. General scheme for the bioactivation of some pyrrolizidine alkaloids. Asterisks (*) denote asymmetric carbon atoms; Nu denotes nucleophile.

alkaloids depend on the reduction back to their parent compounds -- a reaction which has been shown to occur in the gut of the rat (21) and in the rumen of sheep (2). Both the C-7 and C-9 of the dehydroalkaloids are highly electrophilic, especially when they bear an acyloxy grouping. However, under slightly acid conditions, the C-7 and C-9 dehydroalkaloids are also strongly electrophilic when they bear a hydroxyl group. The dehydroalkaloids thus are potentially bi- or mono-functional alkylating agents which may readily react with nucleophilic constituents in the cell or may react with water to yield more stable dehydroaminoalcohols, such as dehydroheliotridine and dehydroretronecine (secondary pyrrolic derivatives).

Esters of otonecine (e.g., senkirkine, hydroxysenkirkine, petasitenine and clivorine) also yield reactive pyrrolic metabolites by initial N-demethylation, to give an 8-hydroxypyrrolizidine, and then the dehydroalkaloid of the corresponding ester.

Strong evidence has pointed to the pyrrolic metabolites as the major metabolites involved in the hepatotoxic, mutagenic and carcinogenic activities of pyrrolizidine alkaloids. However, it is not known whether either or both the dehydroalkaloids and the dehydroaminoalcohols are proximate carcinogens. Black and Jago (95) demonstrated that dehydroheliotridine, the major pyrrolic metabolite of heliotrine and lasiocarpine, can interact with calf thymus DNA in vitro. Alkylation of DNA by dehydroretronecine has also been shown in vitro (32, 96-98) and in vivo (32, 99). Chemical and spectral analyses have revealed that the major reaction product of dehydroretronecine with deoxyguanosine (dGuo) is 7-(deoxyguanosine-N^2-yl)dehydroretronecine, indicating that the reactive electrophile derived from the protonated dehydroretronecine readily alkylates deoxyguanosine (dGuo) at the N^2 position (98).



Dehydroretronecine deoxyguanosine adduct

Following i.p. administration of monocrotaline to Sprague-Dawley rats, DNA-DNA interstrand cross-links and DNA-protein cross-links are formed in the liver cells. These DNA cross-links have been suggested to be instrumental in the hepatocarcinogenicity of monocrotaline (99).

An alternative hypothesis is that epoxide derivatives may be the hepatotoxic and carcinogenic metabolites of pyrrolizidine alkaloids (5, 100). That the open diester alkaloids are less toxic and carcinogenic than the macrocyclic esters may be explained by steric hindrance of the epoxidation of the C1-C2 double bond by the ester side chain at C-1 in the open ester alkaloids (100).

Recent research shows that jacobine, the pyrrolic derivative of dehydroretronecine, and isobutyryl retronecine, but not retronecine nor the pyrrolic derivative of isobutyryl dehydroretronecine pyrrole, induce gene expression of endogenous avian tumor virus in cultured chick embryo fibroblasts (101).

5.3.2.3.1.5 ENVIRONMENTAL SIGNIFICANCE

Plants containing pyrrolizidine alkaloids are so abundant and widespread that they are found in almost every region of the world. As many as 6,000 species, or 3% of the world's flowering plants are estimated to contain some levels of pyrrolizidine alkaloids (cited in ref. 86). For example, among the 24 species of Heliotropium, currently collected along the border of Mexico and the United States, all contain various amounts of unsaturated pyrrolizidine alkaloids (102). The number of species listed in a recent compilation (86) are believed to be only a small proportion of the pyrrolizidine alkaloid-containing plants which actually exist world-wide. According to Smith and

Culvenor (86), all species in the family of Boraginaceae and the genera Senecio, Crotalaria and Eupatorium should be regarded as potentially hepatotoxic.

Sporadic outbreaks of diseases and death in agricultural livestock and farm animals due to consumption of grass or hay contaminated with pyrrolizidine alkaloid-containing plants have occurred in many parts of the world. Several species, in particular those belonging to the genera of Senecio, Crotalaria and Heliotopium, are classic poisonous plants. Senecio jacobaea (Tansy ragwort or Stinking Willie), for instance, is a common contaminant in pastures and is responsible for the "Pictou disease" of cattle and horses in Canada and the "Winton disease" of livestock in New Zealand (2). Livestock poisoning by consumption of S. jacobaea and other pyrrolizidine alkaloid-containing plants in Australia and the Pacific Northwest of the United States has been a serious problem of considerable economic importance (11).

Human exposure to pyrrolizidine alkaloids may occur through the consumption of plant materials contaminating cereal grains. For example, from 1935 to the mid-1950's, an epidemic of poisoning took place in the U.S.S.R. because of the contamination of bread made from wheat, barley or millet containing Heliotropium lasiocarpum (see 16). During 1974-1975, an outbreak of veno-occlusive disease in northwestern Afghanistan was identified to be due to the consumption of flour made from wheat contaminated with seeds of Heliotropium popovii subsp. gillianum, which contains heliotrine and lasiocarpine (13, 103). Another outbreak of veno-occlusive disease in central India between 1975 and 1976 has been correlated with the ingestion of cereals mixed with seeds of a plant, of the Crotalaria species, containing hepatotoxic pyrrolizidine alkaloids (14). Many incidences of poisoning through consumption of food, contaminated with plants of the genus Senecio, were reported in South Africa (see 3, 16).

Exposure of humans to these alkaloids also occurs as a result of the use of pyrrolizidine alkaloid-containing plants as medicinal herbs. In particular, many species in the genera of Senecio, Heliotropium and Crotalaria are used in countries of Asia, Africa and Europe as herbal remedies for the treatment of a wide range of ailments (see Table XLIII). Cases of human poisoning due to the consumption of these plants or plant extracts for medicinal purposes have been recorded (see 11,16).

In addition, humans may be exposed to pyrrolizidine alkaloids through food products since several pyrrolizidine alkaloid-containing plants such as coltsfoot (Petasite japonicus) or comfrey (Symphytum officinale) have been used in Japan, Australia, Europe and the United States as vegetables and for the preparation of herb teas (see Table XLIII). Furthermore, small amounts of pyrrolizidine alkaloids have been detected in the milk of tansy-fed cows (104) as well as in honey produced from the nectar of Senecio jacobaea (105) and Echium plantagineum (106).

So far, there is no epidemiologic evidence that links pyrrolizidine alkaloids with carcinogenesis in humans. However, the high incidence of liver cancer in the Bantu population of South Africa has been related to the use of Senecio plants for medicinal and other purposes (18). The desert Bedouins in Kuwait, who use Heliotropium ramosissimum (which contains heliotrine) as an herbal remedy and for certain other purposes, have a higher incidence of liver cancer than do town dwellers (15).

5.3.2.3.2 Plant Alkaloids Other Than Pyrrolizidine

5.3.2.3.2.1 INTRODUCTION

In addition to pyrrolizidine alkaloids, several other plant alkaloids have received considerable attention because of their therapeutic and pharma-

cologic actions. The medicinal use of plants containing reserpine, sanguinarine, emetine and quinine in various parts of the world dates back almost to antiquity. Despite the advent of synthetic drugs, reserpine and quinine are still prescribed presently for the treatment of hypertension and malaria, respectively. Recent research has discovered that vinblastine, vincristine, acronycine and emetine are effective against certain neoplasms and are valuable agents in cancer chemotherapy. Nicotine and caffeine have no important clinical application; however, their pharmacological actions have been well established. Experimental studies indicate that several of these plant alkaloids are genotoxic and teratogenic. The possibility that they may produce neoplasms in animals and humans has also been investigated. A number of general reviews on these alkaloids has appeared (1, 107-114).

5.3.2.3.2.2 PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL EFFECTS

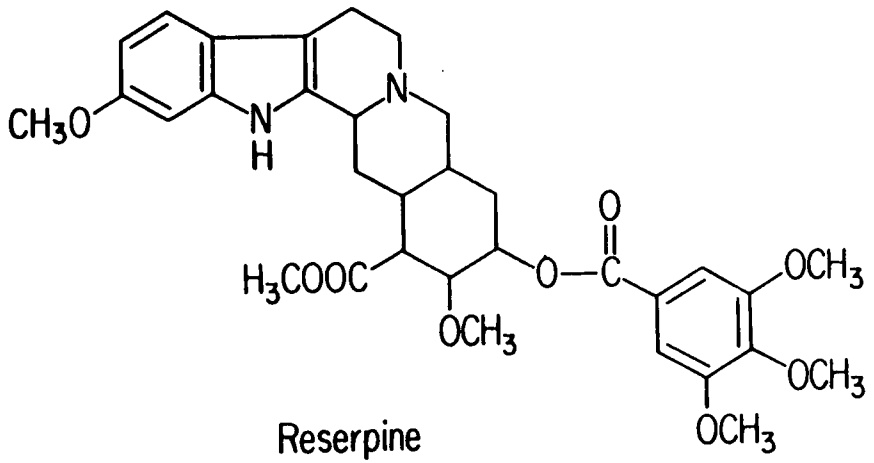
5.3.2.3.2.2.1 Physical and Chemical Properties. The structural formulas of some plant alkaloids tested for carcinogenic activity are shown in Table LI. Like many other alkaloids, they contain nitrogen atom(s) within a heterocyclic ring system. Except for caffeine, which is a xanthane derivative, others are biosynthesized from amino acids and are basic. Nicotine is one of the few liquid alkaloids; others are colorless crystalline solids with well defined melting points. They are highly susceptible to decomposition by heat or light; however, salification with inorganic acids increases their stability. Some important physicochemical properties of these alkaloids are given in Table LII.

5.3.2.3.2.2.2 Biological Effects Other Than Carcinogenicity

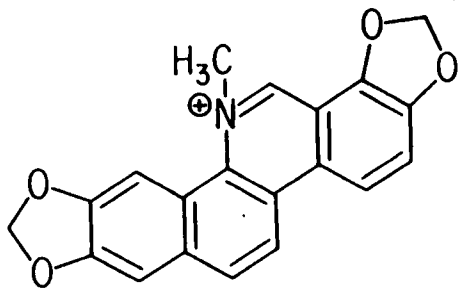
Pharmacological effects. For decades, many of these alkaloids received much more attention from pharmacologists than from toxicologists. The phar-

Table LI

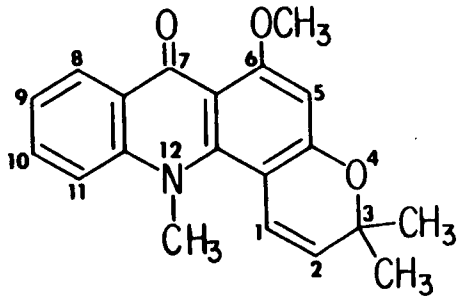
Some Plant Alkaloids Which Have Been Tested for Carcinogenic Activity



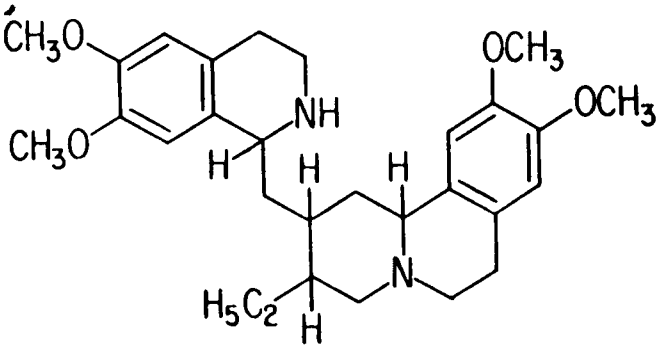
Reserpine



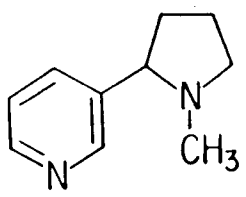
Sanguinarine



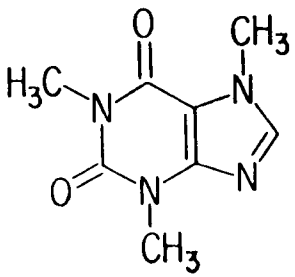
Acronycine



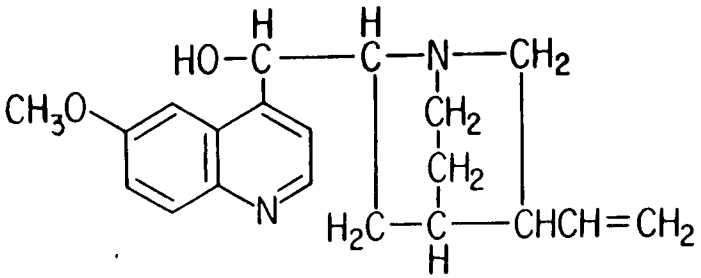
Emetine



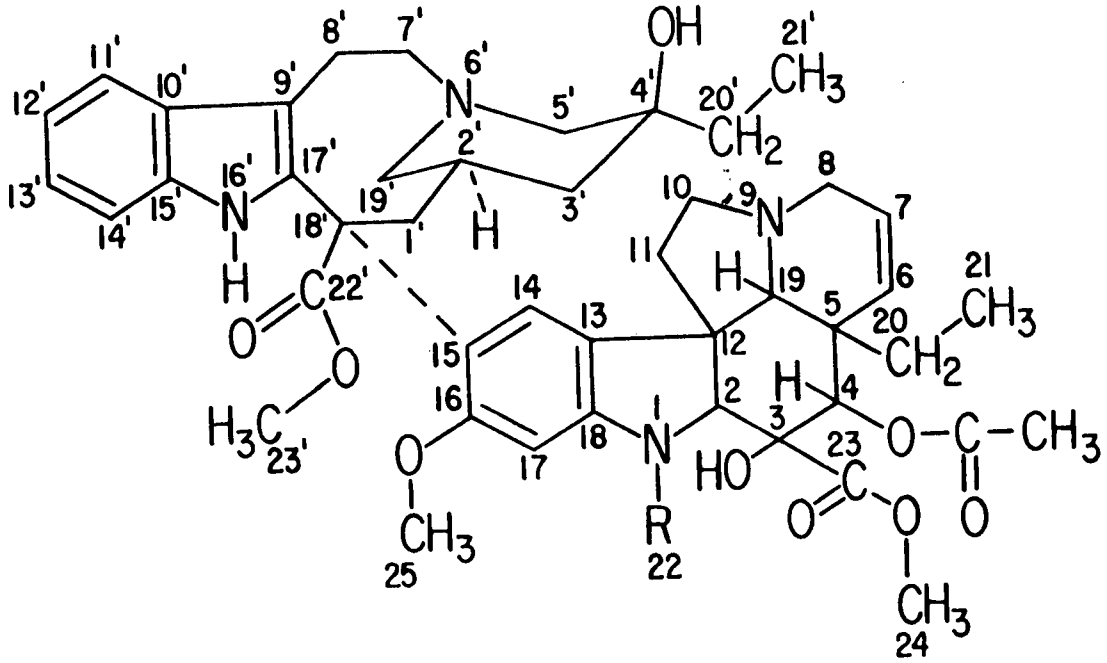
Nicotine



Caffeine



Quinine



Vinblastine R=-CH_3 Vincristine R=-$C(=O)H$

Table LI (p.2 of 2)

Table LII
Physicochemical Properties of Some Plant Alkaloids[a]

Compound[b]	m.p. (°C)	$[\alpha]_D$ (solvent)	Solubility
Reserpine	264-265	-118° (chloroform)	Insoluble in water; soluble in chloroform, dichloromethane, acetic acid, benzene and ethyl acetate; slightly soluble in methanol and ethanol
Sanguinarine	190-191	--	Soluble in ethanol, chloroform, acetone and ethyl acetate
Nicotine (liquid)	--	-169°	Soluble in ethanol, chloroform and ether
Acronycine	--	--	Sparingly soluble in water; soluble in organic solvents
Emetine	74	-50° (chloroform)	Sparingly soluble in water; soluble in chloroform, methanol, ethanol, ether and acetone
Caffeine	238	--	Soluble in water, chloroform, ethanol, acetone and benzene
Quinine	177	-169° (ethanol)	Slightly soluble in water; soluble in ethanol, chloroform, benzene and glycerol
Vinblastine	284-285	-28° (methanol)	Soluble in water and chloroform; slightly soluble in ethane; insoluble in diethyl ether
Vincristine	273-281	+8.5° (methanol)	Soluble in water and chloroform; slightly soluble in ethanol; insoluble in diethyl ether

[a]Summarized from IARC Monographs, Vols. 24 and 26, International Agency for Research on Cancer, Lyon, France, 1980 and 1981; The Merck Index, 10th ed., Merck and Co., Rahway, N.J., 1983.

[b]See Table LI for structural formulas.

macological properties of reserpine (109), quinine (110), nicotine (112) and caffeine (1, 113) have been reviewed. It has become clear that the antihypertensive and sedative effects of reserpine are due to depletion of stores of catecholamines and 5-hydroxytyrosine in the brain and adrenal medulla. The primary antimalarial action of quinine is schizontocidal. In addition, therapeutic doses of quinine cause analgesia and antipyresis and have curare-like effects on skeletal muscle. Nicotine exerts actions on the central and peripheral nervous systems, the cardiovascular system, the gastrointestinal tract and on exocrine glands. In general, the effects of this alkaloid have both stimulant and depressant phases. Five principal pharmacological actions of caffeine are known: respiratory stimulation, skeletal muscle stimulation, diuresis, cardiac stimulation, smooth muscle relaxation, and central nervous system effects.

Toxic effects. Many of these alkaloids are highly toxic to rodents. Their LD_{50} values in the rat, mouse or rabbit are shown in Table LIII.

Undesirable effects of reserpine observed in patients are primarily associated with the gastrointestinal tract and the central nervous system. The following untoward responses are common: abdominal cramps, diarrhea, ulcer, insomnia, nightmares and depression. Single parenteral doses of reserpine produce gastric haemorrhage and erosion (122) and suppress the immune response of lymph node cells (123) in mice.

Sanguinarine is the active poison in argemone seed oil. Epidemics of poisoning from argemone seed oil have been frequently recorded in India. The most common effects include dropsy, diarrhea, edema of the legs, glaucoma, anaemia, fever, redness of the skin, alopecia and dyspnea. All these toxic effects have been experimentally produced in animals with sanguinarine

Table LIII
Acute Toxicity of Some Plant Alkaloids[a]

Compound	Species and route	LD_{50} (mg/kg)	Reference
Reserpine	Rat, i.v.	18	(108)
	Mouse, oral	500	(115)
	i.p.	70	(115)
Sanguinarine	Mouse, i.p.	18	(116)
Nicotine	Rat, oral	55	(117)
	i.v.	1.0	(117)
Emetine	Rat, oral	12.1	(118)
Caffeine	Rat, oral	200	(117)
Quinine	Rabbit, oral	800	(117)
Vinblastine	Rat, i.p.	2.2	(119)
	i.v.	2.9	(120)
	Mouse, oral	15	(119)
	i.p.	5.6	(119)
	i.v.	10	(120, 121)
Vincristine	Rat, i.p.	1.2	(119)
	i.v.	1.0	(120)
	Mouse, i.p.	4.7	(119)
	i.v.	1.7	(121)

[a]See Table LI for structural formulas.

(116). In vitro studies with the pigeon brain homogenates showed that sanguinarine hydrochloride inhibits some -SH group-containing enzymes (124).

Both acronycine and emetine have cytotoxic and antineoplastic properties. Acronycine inhibits the synthesis of nucleic acids by interferring with the transport of nucleosides across the cell membrane (125, 126). Subchronic i.p. administration of acronycine to rats and mice at doses higher than 25 mg/kg resulted in high mortality. At lower doses, inflammation and fibrosis of the peritoneal cavity were noted (127). Emetine is a potent protein synthesis inhibitor in eukaryotes by blocking the transfer of amino acids from t-RNA to the polypeptide chain being formed (128). Structure-activity relationship studies on a number of benzoisoquinoline alkaloids indicate that a planar molecule with 2 aromatic rings and the presence of a nucleophilic element, such as a nitrogen atom at a certain distance from the aromatic rings, are required for protein synthesis inhibitory activity. The distance between the 2 aromatic rings, the angle between the nitrogen atom and the rings, the electronegative character of the rings and the planarity of the structure are important features in determining the activity (129). Clinical toxic manifestations of emetine include nausea, diarrhea, vomiting, epidermal inflammation, aching, tenderness and weakness of muscle and effects on the cardiovascular system (110).

The toxic response of vinblastine and vinicristine are quite different despite their similarity in chemical structures. Vincristine is more toxic in animals and produces, more frequently, peripheral neuropathy, abdominal pain, alopecia and liver impairment. The most important toxic effect of vinblastine is leukopenia. The cytotoxic activity of these agents are related to their ability to inhibit the formation of microtubules of the mitotic spindle, resulting in arrest of dividing cells in metaphase. Structure-activity rela-

tionship analysis reveal that either hydrogenation of the double bonds, reductive formation of carbinols, removal of the acetyl group at C-4 or acetylation of the hydroxyl group diminishes the cytotoxicity of vinblastine and vincristine (111). The configurations at C2' and C18' as well as the presence of the methoxycarbonyl group on C18' (see Table LI) also play an important role in determining biological activity (130).

Quinine is a strong local irritant. The acute toxic effects of quinine in humans are characterized by a spectrum of symptoms referred to as "cinchonism." These symptoms involve hearing and vision, the gastrointestinal tract, the nervous, cardiovascular and renal excretory systems, and the skin. The fatal oral dose of quinine for humans is about 8 grams (110).

Nicotine can be rapidly absorbed through skin and mucous membranes. The major symptoms of acute nicotine poisoning in humans include nausea, vomiting, abdominal pain, diarrhea, cold sweat, headache, mental confusion and convulsions. An oral dose of 40 mg is fatal for an adult. Death may result from respiratory failure caused by paralysis of muscles of the respiratory system.

In humans, overdoses of caffeine (15 mg/kg or more) cause pharmacological responses predominantly in the central nervous system and the circulatory system -- insomnia, restlessness, excitement, sensory disturbances, tachycardia and extrasystoles. Lower doses may cause nausea, nervousness, insomnia and diuresis (113, 117). Caffeine is cytotoxic and affects the mitotic rate of a wide range of cell types (rev. in 107).

Mutagenic effects. The genotoxic potential of these plant alkaloids has been investigated in a number of systems. The results, which are summarized in Table LIV, indicate that many of them possess clastogenic properties but are probably not potent mutagens.

Table LIV
Mutagenic and Related Genotoxic Activities of Some Plant Alkaloids[a]

Compound[b]	Ames test	Chromosomal aberrations	Other test[c]
Reserpine	- (131-133)	+ (143)	- [A-D] (132, 133, 154, 155)
		- (144)	
Sanguinarine[d]	+ (134)		
Vinblastine	- (135, 136)	+ (145)	+ [E,F] (136, 146)
		- (146)	- [D,G-J] (155-158)
Vincristine	- (135)	+ (147)	+ [K,K,L] (149, 159, 160)
		- (148-150)	- [I,J,L] (149, 157, 158, 161)
Quinine[e]	+ (137)	+ (138)	- [F,M,N] (137)
	- (138)		+ [F,L] (138)
Nicotine	- (139-141)	+ (151)	+ [F,L,M] (141, 162)
		- (151)	
Caffeine	- (136, 137, 139, 142)	+ (152, 153)	+ [A,K-M] (107, 151, 163, 164)
			- [D,F,J,K,M,N] (137, 155, 165-169)

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

[b] See Table LI for structural formulas.

[c] A = Bacillus subtilus; B = Aspergillus nidulans; C = Unscheduled DNA synthesis; D = Dominant lethal assay; E = Sperm abnormality test; F = Micronucleus formation; G = Chlamydomonas reinhardi; H = Schizosaccharomyces pombe; I = host-mediated assay; J = Chinese hamster cell/HGPRT assay; K = Mouse lymphoma cell/L5178Y; L = Sister-chromatid exchange; M = Escherichia coli; N = Sex-linked recessive lethal in Drosophila.

[d] Pyrolysate.

[e] Hydrochloride or dihydrochloride salt.

In the Ames Salmonella assay, only the pyrolysate of sanguinarine (134) and the dihydrochloride salt of quinine (137) gave positive results when tested in the presence of S-9 fraction; other alkaloids were negative either with or without metabolic activation (131-133, 135-137, 139-142, 149).

Reserpine was also non-mutagenic in Bacillus subtilis (132) and in Aspergillus nidulans (154). It did not induce dominant lethality in mice (155) or unscheduled DNA synthesis in primary rat hepatocytes (133). Cytogenetic studies on human lymphocytes in culture (132, 151), in Chinese hamster cells in vitro, or in bone marrow cells from rats treated in vivo (132) did not reveal any significant clastogenic activity of reserpine. However, Jameela and Subramanyam (143) have reported that reserpine causes chromosomal aberrations in meiotic cells of grasshoppers and in bone marrow cells of mice.

The genotoxic effects of vinblastine and vincristine have been reviewed (170-172). Most studies showed no mutagenic and related genotoxic activity of these vinca alkaloids. Negative responses were observed for vinblastine in the forward mutation assay in Schizosaccharomyces pombe and in the backward mutation assay in Chamydomonas reinhardi (156). Neither vinblastine nor vincristine displayed mutagenic action in host-mediated assays in rats (149, 157), in dominant lethal assays in mice (155, 156) or in V79 Chinese hamster cells in vitro (158). No increase in chromosomal aberrations (146, 148-150) or in sister chromatid exchange (161) were found by some authors with vincristine in several test systems. Other investigators, however, have reported that vincristine is mutagenic in mouse lymphoma L5178Y cells (159) and induces micronucleus formation (146, 149), sister chromatid exchange (160), chromosomal translocations (173) and other chromosomal aberrations (147). Vinblastine produces increase in chromosomal translocations (173), bone marrow micronucleus formation and sperm abnormalities in mice (136), in addition to

various chromosomal aberrations in cultures of a cell line from the lung of the Chinese hamster (145).

Quinine dihydrochloride is a frameshift mutagen in strains TA98 and TA1538 of Salmonella typhimurium in the presence of S-9 mix, but shows no responses in the following test systems: Escherichia coli, host-mediated assay in mice, sex-linked recessive lethal test in Drosophila melanogaster and micronucleus test in bone marrow cells of mice (137). Quinine hydrochloride, on the other hand, appeared inactive in the Ames test but showed a dose-dependent increase of sister chromatid exchange, enhanced incidence of micronuclei, and elevated chromatid breaks (138).

No mutagenic effect was observed with nicotine in the Ames test (139-141). Cytogenetics studies on human leukocytes in vitro did not disclose any clastogenic activity of nicotine (151). However, the alkaloid induces DNA damage in the E. coli pol A^+/A^- system (141), chromosomal aberrations in mice in vivo (151) and sister chromatic exchange in Chinese hamster ovary cells (162).

No information on the mutagenic activity of acronycine is available. Emetine was reported to exhibit a mutagenic effect when tested in Corynebacterium (cited in 174).

Concern over the genetic hazards involved in the consumption of caffeine-containing beverages have stimulated much research on the mutagenic and related genotoxic activities of caffeine in recent years. The possibility that caffeine might be an environmental mutagen is a subject of many reviews (e.g., 107, 175, 176). Although there exists a series of negative results in a variety of assay systems (e.g., 136, 137, 139, 142, 155, 165-169), the mutagenic and clastogenic actions of caffeine in systems ranging from micro-

organisms and plant cells, to mammalian cells had been established more than twenty years ago (see 107, 175, 176). Recent research has also shown that caffeine induces high frequency of mutations in Bacillus subtilis (163), point mutations and chromosomal breakage in mouse lymphoma cells (153), sister chromatid exchanges in mice in vivo (164), and various types of chromosomal aberrations in somatic ganglia of Drosophila melanogaster (152). Furthermore, a number of studies demonstrate that caffeine has a synergistic effect on the mutagenic and chromosome-damaging activities produced by other chemical mutagens or by radiation (e.g., 177-182). Since caffeine inhibits the activity of DNA polymerase I (183), it is believed that inhibition of excision repair of DNA by caffeine is responsible for its potentiation of mutagenicity.

Structure-activity relationship analysis indicate that caffeine (1,3,7-trimethylxanthine) is more clastogenic in human lymphocytes in culture than 1,3-dimethyl- or 3,7-dimethyl-xanthine; 1,7-dimethyl, 1-methyl-, 3-methyl-, and 7-methyl-xanthines are not clastogenic (184). Caffeine is also more active than its 8-substituted analogs (8-methoxy-, 8-ethoxy-, or 8-chloro-caffeine) as a mutagen in E. coli or as a co-clastogen with thio-TEPA (triethylenethiophosphoramide) or maleic hydrazide in the production of chromosomal aberrations in Chinese hamster cells and Vicia faba root tips (107).

Teratogenic effects. Reserpine is teratogenic in the rat. Spina bifida and eye defects were induced in the offspring of rats administered 0.8-1.5 mg/kg body weight reserpine on day 9, or 1.5-2.0 mg/kg body weight on day 10 of gestation (185). When pregnant rats were parenterally administered 1 mg/kg body weight reserpine daily for 3 days during the last week of gestation, hydronephrosis and deformities of the brain ventricles developed in the newborns (186). In addition, reserpine affects the reproduction and the

postnatal neuroendocrine function of rodents (rev. in 108). In humans, a correlation with malformation has been implicated in a study of 475 pregnant women treated with reserpine and other anti-hypertensive drugs (see ref. 108). There are also reports of nasal congestion with cyanosis, costal retraction, lethargy, congenital lung cysts and stillbirth of babies whose mothers were treated with reserpine during pregnancy (187, 188).

The teratogenic action of antimitotic agents is well documented (see 171, 189). Vinblastine has been shown to be teratogenic in the mouse (190), the rat (191, 192) and the hamster (193). Evidence for the teratogenicity of vincristine has been found also in the monkey (194), the mouse (147, 195), the rat (196) and the hamster (193). In general, both alkaloids induce a similar pattern of effects which include fetal mortality, growth retardation, skeletal defects and malformations of a wide variety of organs. The most effective teratogenic doses of vinblastine and vincristine in these animals are between 0.1 mg/kg and 0.25 mg/kg body weight. In several case reports, no birth defects were found in infants of women receiving various doses of these alkaloids during pregnancy (rev. in 171).

Growth retardation, delay in teeth eruption and in eye opening, and various congenital malformations were noted in the progeny of female rats ingesting quinine (0.25 mg/ml) from drinking water during the pre-gestative, gestative and lactating periods (197). Robinson et al. (198) reported that two of 200 women treated with quinine during early pregnancy gave birth to congenitally deaf babies. In 21 cases of attempted abortion by taking large doses of quinine, congenital malformation involving the central nervous systems, limbs, face and the digestive and urogenital systems resulted (199). Abortion may also result from sanguinarine poisoning (116).

Nicotine, at doses of 0.4, 1.5 and 5.0 mg/kg body weight, has adverse effects on the formation of the cardiovascular system and ossification of the skeleton in embryos of Wistar rats (200). Nishimura and Nakai (201) found skeletal defects and cleft palates in the newborns of mice administered nicotine at 25 mg/kg body weight on days 9, 10 and 11 of gestation.

There was no significant teratogenic response in rats injected i.p. with emetine at 5.0 mg/kg body weight on the 12th day of gestation. However, this inhibitor of protein synthesis potentiates embryolethality and teratogenicity of caffeine and other teratogens (202, 203).

Much information on the teratogenicity of caffeine was obtained from experimental studies in rats, mice, rabbits and hamsters. Thayer and Palm (176) reviewed extensively the teratogenic potential of caffeine and have tabulated the findings reported between 1960 and 1974. The subject has also been covered in update reviews in 1977 (107) and in 1981 (114). Several recent studies confirm most of the earlier observation that at high doses, caffeine is teratogenic in animals. In Charles River CD1 mice, a single dose of 100 mg/kg caffeine injected intraperitoneally on day 14 of pregnancy or single oral doses of caffeine of 200 and 300 mg/kg caused cleft palate in some fetuses (204). Low incidence of retarded skeletal ossification, missing or hypoplastic nails and cleft palate was observed in fetuses of pregnant mice and rats given about 150 mg/kg caffeine in the drinking water (205, 206). Young and Kimmel (207) also noted a dose-related increase in skeletal malformations in the offspring of rats treated intravenously with caffeine on day 11 of gestation at 112.5 mg/kg or 150 mg/kg. Moreover, the potentiating effect of caffeine on the teratogenicity of other agents have been reported (e.g., 202, 208, 209).

Caffeine and its metabolites readily cross the human placenta (210). An increase in the half-life of the excretion of caffeine has been reported in women during pregnancy (211). However, a number of studies which attempted to correlate the intake of caffeine with birth defects in humans showed no definite causal relationship (rev. 114). Nonetheless, an inordinately high incidence of abortion or stillbirth was noted in a subgroup of 16 women during a retrospective survey involving 800 women, three-fourths of whom were Mormons. The 16 women in the subgroup were identified as having an estimated daily intake of caffeine of 600 mg or more (212). A recent nationwide case-control study in Finland, comparing the mothers drinking at least four cups of coffee a day during pregnancy, with those not drinking coffee at all, showed a relative risk of coffee consumption with respect to congential malformation (213).

Several short-term teratogenesis assays have also disclosed the teratogenic action of reserpine, quinine, vinblastine, nicotine and caffeine (214-216).

5.3.2.3.2.3 CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS

5.3.2.3.2.3.1 Carcinogenicity of Plant Alkaloids Other Than Pyrrolizidine. The carcinogenesis studies of some plant alkaloids are summarized in Table LV. Positive carcinogenic effects in experimental animals have been reported with reserpine, sanguinarine, nicotine, acronycine, and most recently with caffeine. In limited studies, no evidence of carcinogenicity was found in rats or mice following chronic exposure to emetine, quinine, vinblastine, or vincristine.

Reserpine. Reserpine is carcinogenic in rats and mice. In a two-year bioassay, in which groups of 50 F344 rats and 50 B6C3F_1 mice of each sex were

Table LV
Carcinogenicity of Some Plant Alkaloids

Compound[a]	Species and strain	Route	Principal organs affected	Reference
Reserpine	Rat, Fischer 344	oral	Adrenal gland	(115)
	Rat, Wistar	oral	Liver, hematopoietic tissue	(217)
	Rat, Wistar	oral	None[b]	(218)
	Mouse, $B6C3F_1$	oral	Seminal vesicle, mammary gland	(115)
	Mouse, C3H; XVIInc	oral	None[c]	(219)
Sanguinarine (or argemone oil)	Rat, --	implantation	Bladder	(116, 220, 221)
	Rat, mouse, hamster and guinea pig	i.v.	(Sarcomas)	(116)
	Mouse, Swiss	topical	Skin	(116, 220, 221)
Nicotine[d]	Rat, Wistar	oral	Intestine, liver	(222)
	Mouse, Swiss	oral	None	(223)
Acronycine	Rat, Sprague-Dawley	i.p.	Mammary gland, bone and peritonium	(127)
	Mouse, $B6C3F_1$	i.p.	None[e]	(127)
Emetine	Rat, Sprague-Dawley	i.p.	None[f]	(224)
	Mouse, $B6C3F_1$	i.p.	None[e,f]	(224)
Caffeine	Rat, Sprague-Dawley	oral	None	(225, 226)
	Rat, Wistar	oral	None	(227)
	Rat, Wistar	oral	Pituitary gland	(228)
	Mouse, C57BL/6	oral	None	(229)

Table LV (continued)

Compound[a]	Species and strain	Route	Principal organs affected	Reference
Quinine	Rat, Leeds	oral	None	(230)
	Mouse, Stock	intravaginal, bladder implantation	None	(231, 232)
Vinblastine	Rat, BR-46	i.v.	None	(233, 234)
	Rat, Sprague-Dawley	i.p.	None	(235)
	Mouse, Swiss	i.p.	None	(235)
Vincristine	Rat, Sprague-Dawley	i.p.	None	(235)
	Mouse, Swiss	i.p.	None	(235)

[a]See Table LI for structural formulas.

[b]Treatment for only 75 weeks.

[c]At a dose level of 0.24 μg/day.

[d]Nicotine pyrolysates or nicotine hydrochloride.

[e]High early mortality rate of treated animals.

[f]Experiment terminated after 84 weeks.

administered reserpine in the feed at doses of 5 ppm or 10 ppm, dose-related neoplasms occurred in both species. Adrenal medullary pheochromocytomas were induced in 24 of the 48 surviving the high-dose, and in 18 of the 49 low-dose male rats. In the mice, 7 cases of mammary carcinomas were found in 48 high-dose as well as in 49 low-dose females; carcinomas of the seminal vesicles, which were not seen in 50 control males, developed in 5 of the 49 high-dose and in one of the 50 low-dose males (115). Low incidences (13-16%) of hepatomas and lymphosarcomas were reported in groups of 43-50 male and 80-92 female Wistar rats receiving reserpine (100 mg/kg) in a semi-liquid diet for 18 months (217). However, administration of 30 or 60 mg/kg body weight of dietary reserpine to groups of 25 male and 25 female Wistar rats for 75 weeks did not result in significant tumor incidence (218). Lacassagne and Duplan (219) detected no tumorigenic activity of reserpine in a group of 24 female C3H mice and in a group of 11 female XVIInc mice, receiving an average of 0.24 µg reserpine per day in the diet for life. The failure of the last two experiments to confirm the carcinogenic activity of reserpine may have been due to the short exposure period and the low dose used.

Sanguinarine. Hakim (116, 220, 221) has established in several experiments that sanguinarine and its major metabolite, benz[c]acridine, are complete carcinogens, inducing bladder tumors in rats and skin tumors in mice. The author induced tumors of the bladder by implanting paraffin pellets (15 mg) containing 25% sanguinarine or benz[c]acridine into the bladder of rats. Skin tumors were induced by repeated painting with solutions of sanguinarine or benz[c]acridine on the skin of mice. Metastasizing sarcomas have also been produced in rats, mice, hamsters and guinea pigs with a single i.v. injection of 0.05 to 0.1 ml argemone oil (containing about 0.1 mg sanguinarine). The carcinogenicity and structure-activity relationships of benzacridines have been discussed in Section 5.1.1.4, Volume IIA.

Nicotine. Truhaut and DeClercq (222) have described the development of malignant teratomas and chronic inflammatory lesions in the intestine and liver of some Wistar rats 12-17 months following ingestion of nicotine or nicotine pyrolysates in the drinking water at a dose level of 10 mg/kg body weight. Control animals or rats s.c. injected with 5 mg/kg nicotine pyrolysate once weekly for life did not bear such tumors. The authors and associates (236, 237) further showed that cotinine (see Fig. 12 for structural formula), the major metabolite of nicotine, is also carcinogenic inducing lymphoid sarcomas or lymphoid leukemia in the alimentary tract, the liver, the lung and the spleen in 12 of 15 rats given cotinine (500 mg/liter) in the drinking water for 8-18 months. Schmahl and Osswald (238), however, failed to confirm the carcinogenic action of cotinine in a group of 100-day-old Wistar rats given approximately 30 mg/kg body weight of cotinine in the drinking water for 17-21 months. Administration of nicotine hydrochloride to groups of 50 male and 50 female Swiss mice (5-6 weeks old) in the drinking water at a concentration of 0.0625% or 0.09375% for life, did not produce a significant tumor incidence (223). There was also no tumorigenic effect of nicotinic acid (pyridine-β-carboxylic acid), a vitamin that occurs in legumes, corn and other plants, under similar study conditions (223).

Nicotine sulfate, which was used together with copper sulfate as a drench to combat parasites on a large farm in South Africa from 1952 to 1962, was incriminated in the high incidence of esophageal tumors in sheep (239).

Acronycine. Acronycine is carcinogenic in Sprague-Dawley rats, inducing tumors of the mammary gland in females, osteosarcomas in males, and sarcomas and other tumors of the peritoneum in both sexes. These findings arose in a chronic study in which groups of 35 rats of each sex were administered acronycine 3 times weekly by i.p. injection at a dose of 3.75 mg/kg body weight

for about one year. In groups of $B6C3F_1$ mice, receiving either 2, 6, 12.5 or 25 mg/kg acronycine, the high mortality rates precluded an evaluation of the carcinogenic effect of acronycine in this species (127). The mouse pulmonary tumor assay, in which a total dose of 0.5, 1.3 or 2.6 g/kg acronycine was given to A/He mice in 5 i.p. injections over 8 weeks, did not reveal the carcinogenic effect of acronycine (240).

Emetine. The carcinogenic potential of emetine has been studied in Sprague-Dawley rats and $B6C3F_1$ mice by administering the alkaloid via i.p. injection at doses of 0.5 or 1 mg/kg body weight for rats and 1.6, 3.2 or 6.4 mg/kg body weight for mice 3 times/week for up to 52 weeks. At the termination of the studies (at week 83 or 84), no tumors occurred at a statistically significant incidence in treated rats or mice compared with controls. However, it was noted that the survival of the treated mice was low in this study. The study was conducted only for 84 weeks instead of two years (224).

Emetine did not exhibit a positive response in the mouse pulmonary tumor assay at the dose levels of 40, 100 and 185 mg/kg body weight (240).

Caffeine. Caffeine was found to be non-tumorigenic in animals by several investigators. No significant increase in tumor incidence was found in mice or rats given caffeine in the diet (225, 229) or in the drinking water (226, 227) for up to two years, at doses exceeding the maximum tolerated level. Similarly, oral administration of freshly brewed or instant coffee to mice or rats for life did not result in higher incidence of tumors in various organs (241-244). However, Yamagami et al. (228) reported in 1983 that caffeine caused pituitary tumors in female Wistar rats. Microadenomas, papillary (or sinusoidal) macroadenomas, and diffuse macroadenomas or hyperplasia of the pituitary were found in 27 of the 40 rats receiving caffeine at a concentra-

tion of 2 mg/ml in the drinking water for 12 months. Such lesions were found only in 9 of 30 control rats.

Quinine. There is no evidence that quinine is carcinogenic. Early experiments conducted by Boyland and associates (231, 232) did not yield significant incidence of neoplasms of the uterine cervix or the bladder in mice following application of quinine sulfate intravaginally or by implantation of the compound in the urinary bladder. More recently, the effects of chronic oral dosing with quinine sulfate in the rat have been examined by Flaks (230). A group of 48 male albino rats of Leeds strain received 0.1% quinine sulfate in the drinking water for up to 15 months. No tumors or preneoplastic lesions were noted in the rats autopsied during the course of the experiment or in the 13 survivors after 15 months of treatment.

Vinblastine. Vinblastine did not produce significant tumor incidence in rats or mice when tested by i.v. or i.p. administration. In a group of 36 male BR46 rats given i.v. injections of 0.33 mg/kg body weight (17% of the LD_{50}) vinblastine sulfate once every two weeks for 10 weeks, a 12% tumor incidence was found. However, 11% had tumors among the 689 controls (233, 234). In another study, in which 48 male BR46 rats were injected intravenously with 0.14 mg/kg body weight vinblastine sulfate once weekly for 52 weeks, only one of the 25 surviving rats bore a benign thymoma 18 months after start of treatment (233). When groups of 25 Sprague-Dawley (CD) rats and 25 Swiss-Webster mice of either sex were administered the compound by i.p. injection, 3 times weekly for 26 weeks at doses of 0.1 and 0.2 mg/kg body weight (rats) or 0.09 and 0.18 mg/kg body weight (mice), the tumor frequencies in the treated animals were not statistically different from the control values (52% vs. 34% in male rats; 72% vs. 58% in female rats; 16% vs. 26% in male and female mice) (235).

Vincristine. As with vinblastine, there is no evidence for carcinogenic activity in rats and mice after i.p. administration of vincristine. The tumor incidences in groups of 25 Sprague-Dawley (CD) rats and 25 Swiss-Webster mice of each sex, given i.p. injections of vincristine sulfate at 0.06 and 0.12 mg/kg body weight (to rats) or 0.075 and 0.15 mg/kg body weight (to mice) 3 times/week for 26 weeks, were not significantly higher than those in the controls (235). The compound did not produce morphological transformation of mouse C3H/10$T_{1/2}$ clone 8 cells (148) or hamster embryo cells (245).

5.3.2.3.2.3.2 Modification of Carcinogenesis. Experimental studies in animals have demonstrated both enhancing and protecting effects of plant alkaloids upon carcinogenesis with other chemicals, depending on the time of treatment and dose level.

Reserpine, for instance, stimulated the induction of mammary tumors by 7,12-dimethylbenz[a]anthracene (DMBA) or by N-nitrosomethylurea (NMU) in the rat, when it was given after the administration of the carcinogen; however, when reserpine was administered before or concurrently with DMBA or NMU, it suppressed mammary tumorigenesis (246, 247). Retardation by reserpine of hepatocarcinogenesis by diethylnitrosamine in rats (248) and of mouse skin tumorigenesis by 3-methylcholanthrene (249) was observed when the animals were treated with reserpine and the carcinogen simultaneously.

Nicotine has long been suspected to be one of the cocarcinogens in cigarette smokers. In studies using the two-stage mouse skin model, nicotine promotes the tumorigenesis of DMBA. It enhances, at dose levels between 2.5 and 5.0 mg/kg body weight, and inhibits, at higher doses, the carcinogenic activity of mixtures of benzopyrene and 12-O-tetradecanoylphorbol-13-acetate (TPA) on the mouse skin (250). A tumorigenesis-promoting activity has also

been observed with cotinine but not with nicotine-1'-N-oxide. It was suggested that metabolism of nicotine to cotinine might account for the cocarcinogenic effect and metabolism to nicotine-1'-N-oxide might account for the inhibitory effects of nicotine at high doses (250). Nicotine has also been shown to enhance stomach carcinogenesis by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the rat; combined treatment of rats with MNNG and nicotine led to an earlier development and an increased incidence of stomach tumors (251).

Welsch et al. (252) observed that the administration of caffeine in the drinking water (250 and 500 mg/l) to female Sprague-Dawley rats, 3 days after treatment with DMBA (5 mg, i.g.) for 21 weeks, resulted in an increase in mammary carcinoma incidence. The same effect was found when caffeine was given to the rats for 6 weeks beginning 20 weeks after exposure to DMBA. However, administration of caffeine prior to and during DMBA treatment did not significantly affect mammary tumor incidence in the rats. On the other hand, caffeine markedly increased the incidence of papillomas on the skin of mice, when it was given 6-9 hours before urethan administration. Application of caffeine to the skin of mice 6 hours after urethan, however, led to lower incidence of skin tumors (253). Nomura (254) found that when lung tumors were induced in young adult mice or in mouse fetuses by s.c. injection of either urethan or 4-nitroquinoline-1-oxide, the tumor incidences were significantly reduced by caffeine after treatment with the carcinogen. The caffeine-sensitive period for suppression of lung neoplasia was found to parallel the generation time of the stem cells in the lung of mouse fetuses and young adult mice. It has been suggested that the inhibition by caffeine of the error-prone post-replication repair of DNA or the alteration of the metabolism of carcinogens by caffeine may be the mechanism of the generally observed modification of chemically-induced neoplasia by caffeine (253, 254). The effects of

caffeine on the hepatic mixed-function oxidases in various species have been described (255).

Ranadive et al. (256) observed, in the mouse, the cocarcinogenic effect of argemone-oil (which contains sanguinarine as the principal constituent) and of a market sample of mustard oil from India.

Recent research indicates that many chemicals, which induce viral gene expression in human lymphoblastoid cells latently infected with Epstein-Barr virus (EBV), are mouse skin tumorigenesis promotors. Although vinblastine possesses EBV-activating property, it fails to show any significant tumorigenesis-promoting activity in the mouse skin (257).

5.3.2.3.2.4 METABOLISM AND POSSIBLE MECHANISMS OF ACTION

The pharmacokinetics and metabolic fate of many of these alkaloids have been investigated in various laboratory animals as well as in humans. Except for sanguinarine and nicotine, metabolic activation does not appear to be required for their biological and carcinogenic activities.

Reserpine. Reserpine is rapidly absorbed and metabolized in most tissues of rats, mice, dogs and rhesus monkeys. In the rat, reserpine is hydrolyzed to methyl reserpate which is excreted primarily in the urine (258). In the mouse, the major urinary metabolites after oral or i.v. administration of reserpine is trimethoxybenzoic acid (259).

As reserpine has not been shown to be mutagenic, a possible mechanism by which reserpine exerts its mammary carcinogenic effects may be via certain endocrine functions. In rodents, reserpine administration has been demonstrated to be associated with elevated levels of serum prolactin and it is known that a correlation exists between the duration and extent of increase in prolactin levels and the development of mammary neoplasms (252, 260).

Recently, it has been shown that chronic reserpine treatment increases the mammary tumor estrogen receptor and peroxidase activity in the rat, indicating that there is increased estrogenic stimulation of specific protein production (247). Furthermore, reserpine has been shown to impair the immune system of rats, inhibit oxidative phosphorylation in isolated mitochondria, and to interfere with transport systems in the cell membrane (see ref. 261).

It is interesting to note that unlike reserpine, yohimbine, another indole alkaloid, which differs from reserpine in lacking an acetyl group and the trimethoxybenzoic moiety in the molecule, does not modify chemically induced carcinogenesis in the liver of the rat (262).

Sanguinarine. In the rat, sanguinarine is readily absorbed after feeding, is stored in the liver, and is biotransformed to at least four different fluorescent metabolites which are excreted as protein complexes in milk, bile and urine. Following parenteral administration into five species of animals, this carcinogenic alkaloid distributes into the stomach and esophagus where metabolism occurs. One of the metabolites has been identified as benz[c]acridine (116). The carcinogenic activity of sanguinarine and benz[c]acridine may be related to their intercalation into DNA (263, 264; see also Notes to Section 5.1.1.6.2.3, Volume IIA).

Nicotine. Nicotine is well absorbed through the respiratory tract, intestine and skin. Radioactivity was detectable in the bronchial wall, the melanin-containing tissues and in the urinary bladder wall, for up to a month after i.v. injection of ^{14}C-methyl- or 2'-^{14}C-labelled nicotine into the mouse (265). The compound is metabolized mainly in the liver but also in the lung and kidney. The major metabolites are cotinine, formed by oxidation at the α-carbon, and nicotine-1'-N-oxide, formed by N-oxidation of the pyrrolidine

ring (266). N-Demethylated metabolites and CO_2 are produced in the lung (267-269). Nicotine and its metabolites are mainly excreted through the kidney; they have also been detected in the milk of lactating women who smoke (270).

Since both carcinogenic and cocarcinogenic activity have been shown for cotinine (see previous Sections), the biotransformation of nicotine to cotinine appears to be important for the carcinogenic action of nicotine. It has been postulated (271) that oxidative N-demethylation of nicotine (or cotinine) may yield chemically active N-methyleniminium species that can interact with cellular nucleophiles (see Fig. 12).

Acronycine. Metabolic studies of acronycine in rats, mice, dogs, cats, rabbits, guinea pigs and humans have shown that hydroxylation of the compound at C-9 and C-11 (see Table LI) occurs in all species. The gem-dimethyl groups (C-3) of acronycine are hydroxylated in rats, mice, dogs and humans but not in guinea pigs. The mouse and the guinea pigs also metabolize acronycine by O-demethylation (272).

The mechanism(s) of the carcinogenic action of acronycine is obscure. It is not known if its interaction with cell-surface components and interference with some transport systems on the cell membrane (125, 126) may result in impaired cellular activities leading to alterations of gene expression. On the other hand, acronycine and its metabolites may act by intercalation with DNA by virtue of the acridine moiety in the molecule.

Emetine. Following parenteral administration, the alkaloid can be found in the liver and, to lesser extent, the lung, kidneys and spleen of humans. Emetine is metabolized slowly; considerable levels of the compound can still be found in the urine 40 to 60 days after treatment (110).

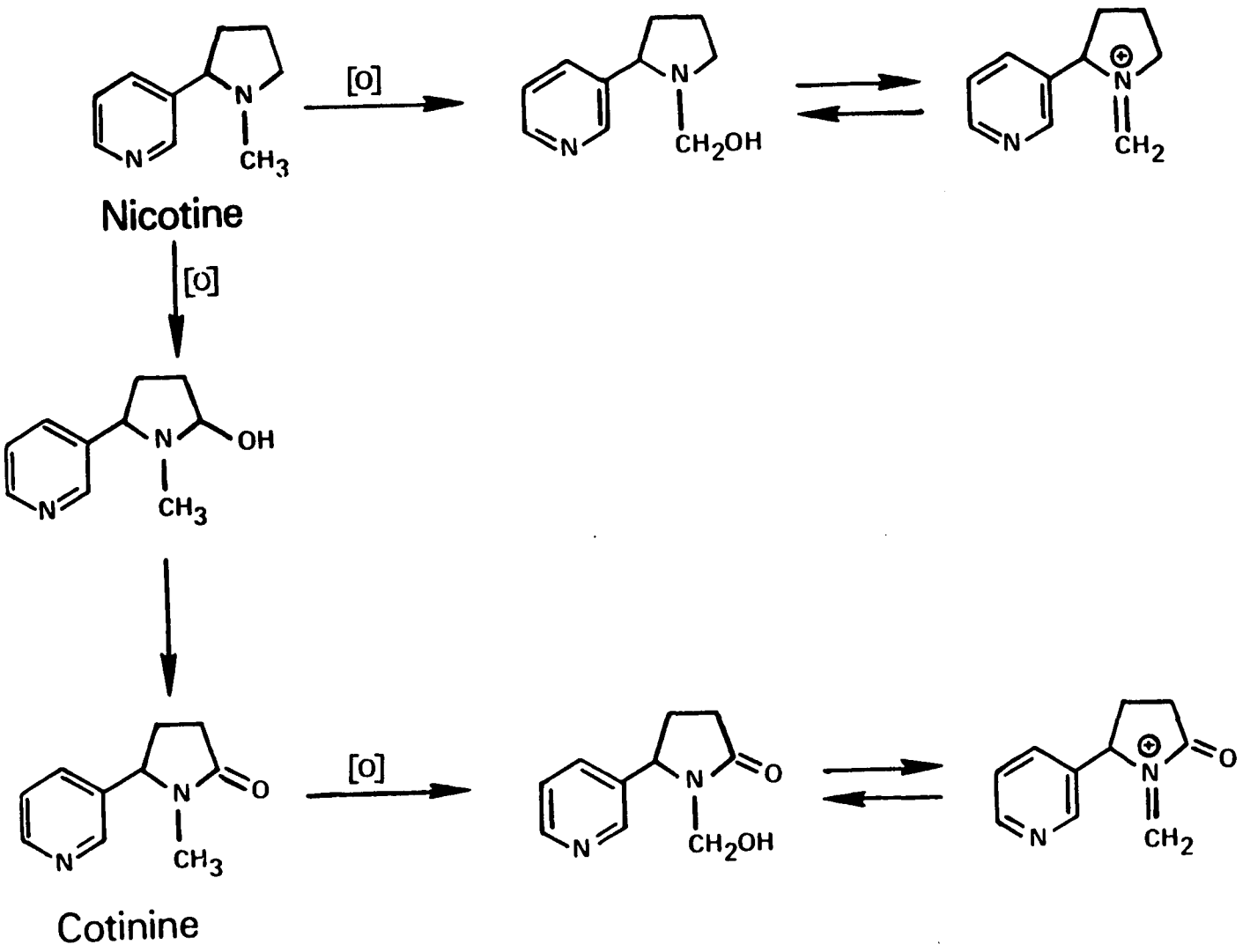


Fig. 12. Proposed metabolic pathway for the activation of nicotine.

Caffeine. The pharmacokinetics and metabolism of caffeine in humans and animal species have been reviewed (107, 114). Recent investigations (273, 274) confirm that the major pathways in the metabolism of caffeine in humans are N-demethylation and ring oxidation to paraxanthine (1,7-dimethylxanthine), theophylline, theobromine and 1,3,7-trimethyluric acid. These compounds are further degraded to dimethylated uric acids, monomethylated uric acids, and monomethylxanthines. An acetylated metabolite, 5-acetylamino-6-amino-3-methyluracil, has also been detected in the urine of humans after oral uptake of caffeine (274, 275). In the mouse, the major urinary metabolites of caffeine are 1,3,7-trimethyluric acid, 1,3-dimethyluric acid, 1-methyluric acid, 1,7-dimethylxanthine, 3-methylxanthine and 6-amino-5-(N-formylmethyl-amino)-1,3-dimethyluracil (114, 276). Metabolism of caffeine in rats also involves hydrolytic ring-opening, resulting in the formation of diaminouracil derivatives up to 30-40% of recovered metabolites (277).

Despite its genotoxic action in some test systems, caffeine does not bind covalently to DNA from perfused liver of the rat (278). The inhibition of post-replication repair of DNA has been suggested to be related to the induction of rat pituitary tumors by caffeine (228).

Quinine. Quinine is rapidly absorbed in the small intestine and is largely metabolized in the liver. The metabolic products, many of them identified as hydroxy derivatives, are excreted in the urine and to a lesser extent in the feces, bile and saliva (110).

Vinblastine and Vincristine. The metabolism of these two vinca alkaloids in various animal species (171) and in humans (111) have been reviewed. They are distributed to most tissues in rats and mice following parenteral administration. Appreciable amounts of these alkaloids are excreted unchanged,

indicating that they are not metabolized to a great extent in rodents (279). Metabolism of vinblastine and vincristine in humans involves only alteration of side chains but not the ring system itself (280, 281). In humans (280) as well as in dogs (282), one of the metabolic products of vinblastine is deacetylvinblastine.

5.3.2.3.2.5 ENVIRONMENTAL SIGNIFICANCE

For centuries, humans have been exposed to many of these alkaloids since they occur in a wide variety of plants which serve as raw materials for the preparation of a number of medicinal agents, edible oils, stimulating beverages and tobacco products. The botanical sources and uses of these alkaloids are presented in Table LVI. Long-term exposure to reserpine, sanguinarine, caffeine or nicotine has been suspected to be the cause of several human cancers.

Reserpine. The therapeutic uses in India of extracts of Rauwolfia serpentia in the treatment of hypertension, insomnia, insanity and snake-bite dates back many centuries. The root of the plant has long been known in traditional Chinese medicine to have an antihypertensive effect. Reserpine, the active agent of the plant, is still an important drug in modern medicine for the treatment of hypertension and psychoses. It has been estimated that millions of people in the United States have used reserpine. In 1974, reserpine was used in some 25% of all cases of diagnosed hypertension in the United States and in 1976 in some 80% of cases in West Germany (see ref. 283).

The possible association between the use of reserpine and the development of breast cancer in women has been a much debated subject. However, the epidemiological data from 14 case-control and 2 cohort studies, involving female populations from various cities in the United States and Europe, do not lead

Table LVI
The Botanical Sources and Uses of Some Plant Alkaloids[a]

Compound[b]	Plants	Uses
Reserpine	*Rauwolfia* spp.	Treatment of hypertension and psychoses
Sanguinarine	*Argemone mexicana*; *Chelidonium maius*; etc.	A medicinal herb in India, China, Africa, and West Indies; adulteration of edible oil
Nicotine	*Nicotina tabacum*; *Duboisia hopwoodii*	A major constituent of tobacco; agricultural insecticide
Acronycine	"Australia scrub ash"	Experimental cancer chemotherapy
Emetine	*Cephaelis impecacuanha*; *C. acuminata*	Treatment of amebic infections; experimental cancer chemotherapy
Caffeine	*Coffea* spp.; *Cola* spp.; *Thea sinensis*; *Paulinia* spp.; *Ilex paraguensis*; *Theobroma Cacao*	An analgesic; preparation of coffee, tea and other beverages
Quinine	*Cinchona officinalis*; *C. succirubrum*; *C. calisaya*; *C. ledgeriana*	Treatment of malaria and nocturnal leg cramps, a bitter-flavored constituent of some carbonated beverages
Vinblastine, Vincristine	*Vinca rosea* (*Catharanthus roseus*); *Catharanthus* spp.	Cancer chemotherapy

[a]Summarized from IARC Monographs Vols. 24 and 26, International Agency for Research on Cancer, Lyon, France, 1980, 1981; A.G. Gilman, L.S. Goodman, and A. Gilman (eds.), "The Pharmacological Basis of Therapeutics," MacMillan, New York, 1980; The Merck Index, 10th ed., Merck and Co., Rahway, N.Y., 1983.

[b]See Table LI for structural formulas.

to an unequivocal conclusion of relationship between reserpine use and breast cancer. Although several studies found a positive relationship, there is only a small increase in risk for long-term users (see 108, 172). This apparent slight increase in risk may even be confounded by socio-economic variables or other breast cancer risk factors (cited in 283). Studies on the effects of reserpine on prolactin level and on the incidence of breast cancer in postmenopausal women suggest that slight increases in prolactin level would not greatly increase the relative risk of breast cancer (284).

A case-control study (285) negates the hypothesis that reserpine exacerbates prostate cancer by stimulating serum prolactin production.

Sanguinarine. Sanguinarine has been found in Argemone mexicana (argemone weed; yellow-flowered prickly poppy) and 49 other plant species belonging to 14 genera of the poppy-fumaria family (Papaveraceae). These plants grow abundantly in both the tropical and the temperate regions of the globe. In India, China, Africa and West Indies, argemone weed is used as a medicine. People in these and other areas are also exposed to sanguinarine through the consumption of edible oils contaminated with argemone oil and/or the intake of milk, liver and eggs from animals which fed on weeds producing sanguinarine. Hakim (161, 221) has noted a correlation between the geographical distribution and density of sanguinarine-producing plants and the local incidences of oesophageal cancer and stomach cancer. The high incidence of nasopharyngeal cancer in some Chinese and Pillipinos populations is suspected to be related to the smoking of opium which also contains sanguinarine (221).

Nicotine. Nicotine is one of the alkaloids to which humans are most frequently exposed, since it is a major constituent of tobacco Nicotiana tabacum and Duboisia hopwoodii. The very strong statistical association of lung

cancer and cigarette smoking is well established. It is also known that tobacco ingredients are initiators and/or promotors of carcinogenesis. Although the principal carcinogenic and co-carcinogenic agents in tobacco smoke are polycyclic aromatic hydrocarbons (see ref. 286), it is possible that nicotine may play the role of a cocarcinogen and/or promotor. The tobacco specific nitrosamines, N'-nitrosonornicotine and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, which have been found to be carcinogenic in rodents (see Section 5.2.1.2, Volume IIIA), are derived from nicotine.

In the United States, a large quantity of nicotine sulfate was used as an agricultural insecticide before being replaced by other chemicals.

Acronycine and Emetine. In experimental and clinical studies, both alkaloids have been demonstrated to be effective agents against a broad spectrum of tumors. Acronycine is obtained from the bark of the Australian scrub ash whereas emetine is obtained from ipecac ("Brazil root"), the dried root of Cephaelis ipecacuanha or C. acuminata (127, 224). The emetine-producing plants are native to Central America and Brazil but can also be found in India and Malaysia. Since 1912 emetine is widely used for the treatment of intestinal amebiasis, amebic hepatitis and other severe amebic infections (see 110). A review in 1978 (1) estimated that 11-14 metric tons of ipecac, which contains 12-14% of emetine, is imported into the United States annually, mainly from Brazil.

Caffeine. Caffeine is a constituent of coffee, tea and other beverages. It has a limited use in medicine, mainly as an analgesic and a stimulant (107, 113). Most of the world population is exposed to caffeine to a greater or lesser extent. In 1961, the Expert Panel of the Flavor and Extract Manufacturers' Association of the United States considered caffeine to

be GRAS ("Generally Regarded as Safe") for use in non-alcoholic beverages. However, on October 15, 1980, the U.S. Food and Drug Administration proposed that caffeine be no longer listed as GRAS, on the basis of more recent research data (see 114).

A number of epidemiologic studies on the carcinogenic and teratogenic effects of caffeine consumed in coffee and tea are available (revs. 114, 287). Stocks (288) compared the age-adjusted death rates due to cancer at various sites and the annual consumption of coffee and tea in 20 countries during 1964-1965. The author concluded that consumption of coffee positively correlates with leukemia and cancer of the pancreas, prostate and ovary. A positive correlation also exists between the consumption of tea and cancer of the intestine, larynx, lung and breast. However, Heyden (289) found several flaws in this study. Cole (290) found an association between coffee-drinking and bladder cancer in a case-control study. Later studies from the same author (291) and from other investigators (292), however, failed to substantiate the observed association. Shennan (293) reported a strong correlation between coffee consumption and the rates of mortality from renal cancer in 16 countries. On the other hand, studies by others (294, 295) did not find such association. More recently, MacMahon et al. (296) drew the attention to a strong correlation between coffee consumption and pancreatic cancer in both men and women. Review of the available data shows only ambiguous evidence for reproductive or teratogenic effects due to ingestion of caffeine from coffee (see 114).

Quinine. Quinine is the principal alkaloid of cinchona, the dried bark of various Cinchona species. The history of this antimalarial agent dates back for more than 300 years. In fact, quinine was the sole remedy for malaria until World War II. This agent is still used for the management of

malaria and for the relief of nocturnal leg cramps (110). It also finds use as a bitter-flavored constituent of a widely used type of carbonated beverage (230).

Vinblastine and Vincristine. In folk medicine, these two alkaloids from the periwinkle plant (Vinca rosea) were used for controlling hemorrhage, for the treatment of scurvy and toothache, and for healing chronic wounds. Since the early 1960's, they have proved to be important agents, either singly or in combination with other antineoplastic drugs, in the therapy of Hodgkins disease, lymphosarcomas, choriocarcinomas, testicular tumors and other forms of human neoplasms (1, 111, 297). Several case reports and epidemiologic studies on the carcinogenic activity of these two drugs in humans have been reviewed (171). In chemotherapy, with drug combinations including vincristine or vinblastine, the two agents have been associated with the subsequent development of leukemias.

REFERENCES TO SECTION 5.3.2.3

1. Cordell, G.A.: Kirk-Othmer Encycl. Chem. Technol. (3rd ed.), 1, 883 (1978).
2. Bull, L.B., Culvenor, C.C.J., and Dick, A.T.: "The Pyrrolizidine Alkaloids. Their Chemistry, Pathogenicity and Other Biological Properties." Wiley, New York, 1968, 293 pp.
3. McLean, E.K.: Pharmacol. Rev. 22, 429 (1970).
4. Miller, J.A.: Naturally Occurring Substances that can Induce Tumors. In "Toxicants Occurring Naturally in Food" (2nd ed.), National Academy of Sciences, Washington, D.C., 1973, p. 508.
5. Schoental, R.: Carcinogens in Plants and Microorganisms. In "Chemical Carcinogen" (C.E. Searle, ed.), ACS Monogr. No. 173, Chapter 12. Am. Chem. Soc. Washington, D.C., 1976, p. 626.

6. Culvenor, C.C.J., and Jags, M.V.: Carcinogenic Plant Products and DNA. In "Chemical Carcinogens and DNA" (P.L. Grover, ed.), Chapter 6, CRC Press, Boca Raton, Florida, 1979, p. 161.

7. Hirono, I.: CRC Crit. Rev. Toxicol. 8, 235 (1981).

8. IARC: "Some Naturally Occurring Substances," IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 10, International Agency for Research on Cancer, Lyon, 1976, 353 pp.

9. International Agency for Research on Cancer: IARC Monogr. 31, 231 (1983).

10. International Agency for Research on Cancer: IARC Monogr. 31, 239 (1983).

11. Huxtable, R.J.: Gen. Pharmac. 10, 159 (1979).

12. Culvenor, C.C.J., Edgar, J.A., Jago, M.V., Outteridge, A., Peterson, J.E., and Smith, L.W.: Chem.-Biol. Interact. 12, 299 (1976).

13. Mohabbat, O., Srivastava, R.N., Younos, M.S., Sediq, G.G., Merzad, A.A., and Aram, G.N.: Lancet 2, 269 (1976).

14. Tandon, B.N., Tandon, R.K., Tandon, H.D., Narndranathan, M., and Joshi, Y.K.: Lancet 2, 271 (1976).

15. Schoental, R.: Toxicol. Lett. 10, 323 (1982).

16. Culvenor, C.C.J.: J. Toxicol. Environ, Health 11, 625 (1983).

17. Culvenor, C.C.J., Downing, D.T., Edgar, J.A., and Jago, M.V.: Ann. N.Y. Acad. Sci. 163, 837 (1969).

18. Schoental, R.: Cancer Res. 28, 2237 (1968).

19. Mattocks, A.R.: Chem.-Biol. Interact. 5, 227 (1972).

20. White, I.N.H., Mattocks, A.R., and Butler, W.H.: Chem.-Biol. Interact. 6, 207 (1973).

21. Mattocks, A.R.: Xenobiotica 1, 563 (1971).

37. Wehner, F.C., Thiel, P.G., and Van Rensburg, S.J.: Mutat. Res. 66, 187 (1979).

38. White, R.D., Krumperman, P.H., Cheeke, P.R., Deinzer, M.L., and Buhler, D.R.: J. Animal Sci. 58, 1245 (1984).

39. Green, M.H.L., and Muriel, W.J.: Mutat. Res. 28, 331 (1975).

40. Alderson, T., and Clark, A.M.: Nature (London) 210, 593 (1966).

41. Clark, A.M.: Z. Vererb. Lehre. 91, 74 (1960).

42. Candrian, U., Luthy, J., Graf, U., and Schlatter, Ch.: Food Chem. Toxicol. 22, 223 (1984).

43. Cook, L.M., and Holt, A.C.E.: J. Genet. 59, 273 (1966).

44. Takanashi, H., Umeda, M., and Hirono, I.: Mutat. Res. 78, 67 (1980).

45. Williams, G.M., Mori, H., Hirono, I., and Nagao, M.: Mutat. Res. 79, 1 (1980).

46. Craddock, V.M., and Henderson, A.R.: Cancer Res. 38, 2135 (1978).

47. Whitehead, F.W., San, R.H.C., and Stich, H.F.: Mutat. Res. 111, 209 (1983).

48. Sugimura, T., Nagao, M., Kawachi, T., Honda, M., Yahagi, T., Seino, Y., Sato, S., Matsukura, N., Matsushima, T., Shirai, A., Sawamura, M., and Matsumoto, H.: Mutagen-carcinogens in Food, with Special Reference to Highly Mutagenic Pyrolytic Products in Boiled Foods. In "Origin of Human Cancer" (H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1977, p. 1561.

49. Clark, A.M.: Mutat. Res. 92, 89 (1982).

50. Green, C.R., and Christie, G.S.: Br. J. Exp. Pathol. 42, 369 (1961).

51. Newberne, P.M.: Cancer Res. 28, 2327 (1968).

52. Schoental, R.: J. Pathol, Bacteriol. 77, 485 (1959).

53. Cook, J.W., Duffy, E., and Schoental, R.: Br. J. Cancer 4, 405 (1950).

54. Hirono, I., Mori, H., Haga, M., Fujii, M., Yamada, K., Hirata, Y., Takanashi, H., Uchida, E., Hosaka, S., Ueno, I., Matsushima, T., Umezawa, K., and Shirai, A.: Edible Plants Containing Carcinogenic Pyrrolizidine Alkaloids in Japan. In "Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis" (E.C. Miller, J.A. Miller, I. Hirono, T. Sugimura, and S. Takayama, eds.), Japan Scientific Societies (Tokyo)/University Park Press (Baltimore), 1979, p. 79.

55. Schoental, R., Head, M.A., and Peacock, P.R.: Br. J. Cancer 8, 458 (1954).

56. Campbell, J.G.: Proc. Roy. Soc. Edinburgh B. 66 (Pt. 2), 11 (1956).

57. Dybing, O., and Erichsen, S.: Acta Pathol. Microbiol. Scand. 47, 1 (1959).

58. Harris, P.N., and Chen, K.K.: Cancer Res. 30, 2881 (1970).

59. Hirono, I., Ueno, I., Aiso, S., Yamaji, T, and Haga, M.: Cancer Lett. 20, 191 (1983).

60. Schoental, R., Fowler, M.E., and Coady, A.: Cancer Res. 30, 2127 (1970).

61. Schoental, R., Hard, G.C., and Gibbard, S.: J. Natl. Cancer Inst. 47, 1037 (1971).

62. Schoental, R., and Cavanagh, J.B.: J. Natl. Cancer Inst. 49, 665 (1972).

63. Hirono, I., Shimizu, M., Fushimi, K., Mori, H., and Kato, K.: Gann 64, 527 (1973).

64. Hirono, I., Sasaoka, I., Shibuya, C., Shimizu, M., Fushimi, K., Mori, H., Kato, K., and Haga, M.: Gann Monograph Cancer Res. 17, 205 (1975).

65. Fushimi, K., Kato, K., Kato, T., and Matsubara, N.: Toxicol. Lett. 1, 291 (1978).

66. Hirono, I., Mori, H., and Culvenor, C.J.: Gann 67, 125 (1976).

67. Schoental, R.: Cancer Res. 35, 2020 (1975).

68. Svoboda, D.J., and Reddy, J.K.: Cancer Res. 32, 908 (1972).

69. Svoboda, D.J., and Reddy, J.K.: J. Natl. Cancer Inst. 53, 1415 (1974).

70. Rao, M.S., and Reddy, J.K.: Br. J. Cancer 37, 289 (1978).

71. Rao, M.S., Jago, M.V., and Reddy, J.K.: Human Toxicol. 2, 15 (1983).

72. NCI: "Bioassay of Lasiocarpine for Possible Carcinogenicity," NCI Carcinog. Tech. Rep. Ser. No. 39, DHEW Publ. No. (NIH) 78-839. National Cancer Institute, Bethesda, Maryland, 1978.

73. Peterson, J.E., Jago, M.V., Reddy, J.K., and Jarrett, R.G.: J. Natl. Cancer Inst. 70, 381 (1983).

74. Schoental, R., and Head, M.A.: Br. J. Cancer 11, 535 (1957).

75. Schoental, R., and Bensted, J.P.M.: Br. J. Cancer 17, 242 (1963).

76. Schoental, R., and Head, M.A.: Br. J. Cancer 9, 229 (1955).

77. Newberne, P.M., and Rogers, A.E.: Nutrition, Monocrotaline and Aflatoxin B_1 in Liver Carcinogenesis. In "Plant Foods for Man" (P.N. Newman, ed.), 1973, p. 23.

78. Allen, J.R., Hsu, I.-C., and Carstens, L.A.: Cancer Res. 35, 997 (1975).

79. Shumaker, R.C., Robertson, K.A., Hsu, I.-C., and Allen, J.R.: J. Natl. Cancer Inst. 56, 787 (1976).

80. Hayashi, Y., Shinada, M., and Katayama, H.: Toxicol. Lett. 1, 41 (1977).

81. Mattocks, A.R., and Cabral, J.R.P.: Cancer Lett. 17, 61 (1982).

82. Johnson, W.D., Robertson, K.A., Pounds, J.G., and Allen, J.R.: J. Natl. Cancer Inst. 61, 85 (1978).

83. Hirono, I., Mori, H., Yamada, K., Hirata, Y., Haga, M., Tatematsu, H., and Kanie, S.: J. Natl. Cancer Inst. 58, 1155 (1977).

84. Kuhara, K., Takanashi, H., Hirono, I., Furuya, T., and Asada, Y.: Cancer Lett. 10, 117 (1980).

85. Styles, J., Ashby, J., and Mattocks, A.R.: Carcinogenesis 1, 161 (1980).

86. Smith, L.W., and Culvenor, C.C.J.: J. Natural Products 44, 129 (1981).

87. Reddy, J.K., Rao, M.S., and Jago, M.V.: Int. J. Cancer 17, 621 (1976).

88. Hirono, I., Mori, H., and Haga, M.: J. Natl. Cancer Res. 61, 865 (1978).

89. Mattocks, A.R., and Cabral, J.R.P.: Tumori 65, 289 (1979).

90. Schoental, R., and Magee, P.N.: J. Pathol. Bacteriol. 78, 471 (1959).

91. Mattocks, A.R., and White, I.N.H.: Chem.-Biol. Interact. 3, 383 (1971).

92. White, I.N.H., and Mattocks, A.R.: Xenobiotica 1, 503 (1971).

93. White, I.N.H.: Chem.-Biol. Interact. 16, 169 (1977).

94. Mattocks, A.R., and Bird, I.: Chem.-Biol. Interact. 43, 209 (1983).

95. Black, D.N., and Jago, M.V.: Biochem. J. 118, 347 (1970).

96. White, I.N.H., and Mattocks, A.R.: Biochem. J. 128, 291 (1972).

97. Mattocks, A.R., and Bird, I.: Toxicol. Lett. 16, 1 (1983).

98. Robertson, K.A.: Cancer Res. 42, 8 (1982).

99. Petry, T.W., Bowden, G.T., Huxtable, R.J., and Sipes, I.G.: Cancer Res. 44, 1505 (1984).

100. Schoental, R.: Nature (London) 227, 401 (1970).

101. Pearson, M.N., Karchesy, J.J., Deeney, A.O'C., Deinzer, M.L., and Beaudreau, G.S.: Chem.-Biol. Interact. 49, 341 (1984).

102. Birecka, H., Frohlich, M.W., Hull, L., and Chaskes, M.J.: Phytochemistry 19, 421 (1980).

103. Tandon, H.D., Tandon, B.N., and Mattocks, A.R.: Am. J. Gastroenterol. 70, 607 (1978).

104. Dickinson, J.O., Cooke, M.P., King, R.R., and Mohamed, P.A.: J. Am. Vet. Med. Assoc. 169, 1192 (1976).

105. Deinzer, M.L., Thomson, P.A., Burgett, D.M., and Isaacson, D.L.: Science 195, 497 (1977).

106. Culvenor, C.C.J., Edgar, J.A., and Smith, L.W.: J. Agric. Food Chem. 29, 958 (1981).

107. Timson, J.: Mutat. Res. 47, 1 (1977).

108. International Agency for Research on Cancer: IARC Monogr. 24, 211 (1980).

109. Weiner, N.: Drugs That Inhibit Adrenergic Nerves and Block Adrenergic Receptors. In "The Pharmacological Basis of Therapeutics" (A.G. Gilman, L.S. Goodman and A. Gilman, eds.), 6th ed., MacMillan, New York, 1980, p. 176.

110. Rollo, I.M.: Drug Used in the Chemotherapy of Malaria. In "The Pharmacological Basis of Therapeutics" (A.G. Gilman, L.S. Goodman and A. Gilman, eds.), 6th ed., MacMillan, New York, 1980, p. 1038.

111. Calabresi, P., and Parks, R.E. Jr.: Chemotherapy of Neoplastic Diseases. In "The Pharmacological Basis of Therapeutics" (A.G. Gilman, L.S. Goodman and A. Gilman, eds.), 6th ed., MacMillan, New York, 1980, p. 1249.

112. Taylor, P.: Ganglionic Simulating and Blocking Agents. In "The Pharmacological Basis of Therapeutics" (A.G. Gilman, L.S. Goodman and A. Gilman, eds.), 6th ed., MacMillan, New York, 1980, p. 211.

113. Rall, T.W.: Central Nervous System Stimulants. In "The Pharmacological Basis of Therapeutics" (A.G. Gilman, L.S. Goodman and A. Gilman, eds.), 6th ed., MacMillan, New York, 1980, p. 592.

114. Oser, B.L., and Ford, R.A.: Drug Chem. Toxicol. 4, 311 (1981).

115. NCI: "Bioassay of Reserpine for Possible Carcinogenicity," NCI Carcinog. Tech. Rep. Ser. No. 193, DHEW Publ. No. (NIH) 80-1749. National Cancer Institute, Bethesda, Maryland, 1980.

116. Hakim, S.A.E.: Maharashtra Med. J. 17, 109 (1970).

117. Windholz, M. (ed.): "The Merck Index," 10th ed., Merck and Co., Rahway, New Jersey, 1983.

118. Radomski, J.L., Hagan, E.C., Fuyat, H.N., and Nelson, A.A.: J. Pharmacol. Exp. Ther. 104, 421 (1952).

119. Nemeth, L., Somfai, S., Gal, F., and Kellner, B.: Neoplasma 17, 345 (1970).

120. Todd, G.C., Gibson, W.R., and Morton, D.M.: J. Toxicol. Environ. Health 1, 843 (1976).

121. Lu, C.C., and Meistrich, M.L.: Cancer Res. 39, 3575 (1979).

122. Blackman, J.G., Campion, D.S., and Fastier, F.N.: Br. J. Pharmacol. 14, 112 (1959).

123. Devoino, L.V., and Yeliseyeva, L.S.: Eur. J. Pharmacol. 14, 71 (1971).

124. Sarkar, S.N.: Nature (London) 162, 265 (1948).

125. Dunn, B.P., Gout, P.W., and Beer, C.T.: Cancer Res. 33, 2310 (1973).

126. Kessel, D.: Biochem. Pharmacol. 26, 1077 (1977).

127. NCI: "Bioassay of Acronycine for Possible Carcinogenicity," NCI Carcinog. Tech. Rep. Ser. No. 49, DHEW Publ. No. (NIH) 78-849. National Cancer Institute, Bethesda, Maryland, 1978.

128. Grollman, A.P.: Proc. Nat. Acad. Sci. (U.S.A.) 56, 1867 (1966).

129. Gupta, R.S., Krepinsky, J.J., and Siminovitch, L.: Mol. Pharmacol. 18, 136 (1980).

130. Zavala, F., Guenard, D., and Potier, P.: Experientia 34, 1479 (1978).

131. Fonstein, L.M., Revazova, Y.A., Zolotareva, G.N., Abilev, S.K., Akinjshina, L.P., Brazlavsky, V.A., Iskhakova, E.N., Meksin, V.A., Radchenko, L.U., and Shapiro, A.A.: Genetika 14, 900 (1978).

132. Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M., and Sugiyama, T.: IARC Sci. Publ. 27, 323 (1980).

133. Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K., and Neal, S.B.: Environ. Mutagen. 3, 11 (1981).

134. Malaveille, C., Friesen, M., Camus, A.-M., Garren, L., Hautefeuille, A., Bereziat, J.-C., Ghadirian, P., Day, N.E., and Bartsch, H.: Carcinogenesis 3, 577 (1982).

135. Seino, Y., Nagao, M., Yahagi, T., Hoshi, A., Kawachi, T., and Sugimura, T.: Cancer Res. 38, 2148 (1978).

136. Bruce, W.R., and Heddle, J.A.: Can. J. Genet. Cytol. 21, 319 (1979).

137. King, M.-T., Beikirch, H., Eckhardt, K., Gocke, E., and Wild, D.: Mutat. Res. 66, 33 (1979).

138. Munzner, R., and Renner, H.W.: Toxicology 26, 173 (1983).

139. McCann, J., Choi, E., Yamasaki, E., and Ames, B.N.: Proc. Nat. Acad. Sci. (U.S.A.) 72, 5135 (1975).

140. Florin, I., Rutberg, L., Carvall, M., and Enzell, C.R.: Toxicology 18, 219 (1980).

141. Riebe, M., Westphal, K., and Fortnagel, P.: Mutat. Res. 101, 39 (1982).

142. Purchase, I.F.H., Longstaff, E., Ashby, J., Styles, J.A., Anderson, D., Lefevre, P.A., and Westwood, F.R.: Br. J. Cancer 37, 873 (1978).

143. Jameela, M., and Subramanyam, S.: Mutat. Res. 67, 295 (1979).

144. Bishun, N., and William, D.: Lancet 1, 926 (1975).

145. Segawa, M., Nadamitsu, S., Kondo, K., and Yoshizaki, I.: Mutat. Res. 66, 99 (1979).

146. Maier, P., and Schmid, W.: Mutat. Res. 40, 325 (1976).

147. Sieber, S.M., Whang-Peng, J., Botkin, C., and Knutsen, T.: Teratology 18, 31 (1978).

148. Benedict, W.F., Banerjee, A., Gardner, A., and Jones, P.A.: Cancer Res. 37, 2202 (1977).

149. Muller, D., Arni, P., Fritz, H., Langauer, M., and Strasser, F.F.: Mutat. Res. 53, 235 (1978).

150. Au, W.W., Johnson, D.A., Collie-Bryere, C., and Hsu, T.C.: Environ. Mutagen. 2, 455 (1980).

151. Bishun, N.P., Lloyd, N., Raven, R.W., and Williams, D.C.: Acta Biol. Acad. Sci. Hung. 23, 175 (1972).

152. De Marco, A., and Cozzi, R.: Mutat. Res. 69, 55 (1980).

153. Clive, D., Eyre, J., and Hozier, J.: "Caffeine Induces Proportional Frequencies of Small Colony (θ) $TK^{-/-}$ Mutants and Chromosomally Aberrant Cells in L5178Y/$TK^{+/-}$ Mouse Lymphoma Cells." Presented at the 14th Annual Meeting of the Environmental Mutagen Society. Program and Abstracts -- Environmental Mutagen Society, Vol. 14, 77 (1983).

154. Bignami, M., Morpurgo, G., Pagliani, R., Carere, A., Conti, G., and Di Giuseppe, G.: Mutat. Res. 26, 159 (1974).

155. Epstein, S.S., Arnold, E., Andrea, J., Bass, W., and Bishop, Y.: Toxicol. Appl. Pharmacol. 23, 288 (1972).

156. Degraeve, N.: Mutat. Res. 21, 28 (1973).

157. Styles, J.A.: Mutat. Res. 21, 50 (1973).

158. Suter, W., Brennand, J., McMillan, S., and Fox, M.: Mutat. Res. 73, 171 (1980).

159. Matheson, D., Brusick, D., and Carrano, R.: Drug Chem. Toxicol. 1, 277 (1978).

160. Raposa, T.: Mutat. Res. 57, 241 (1978).

161. Morgan, W.F., and Crossen, P.E.: Mutat. Res. 77, 283 (1980).

162. Riebe, M., and Westphal, K.: Mutat. Res. 124, 281 (1983).

163. Sacks, L.E., and Mihara, K.: Mutat. Res. 117, 55 (1983).

164. Nakanishi, Y., and Schneider, E.L.: Mutat. Res. 60, 329 (1979).

165. Brusick, D.J., Simmon, V.F., Rosenkranz, H.S., Ray, V.A., and Stafford, R.S.: Mutat. Res. 76, 169 (1980).

166. Amacher, D.E., Paillet, S.C., Turner, G.N., Ray, V.A., and Salsburg, D.S.: Mutat. Res. 72, 447 (1980).

167. Amacher, D.E., and Zelljadt, I.: Mutat. Res. 136, 137 (1984).

168. Generoso, W., Bishop, J.B., Gosslee, D.G., Newell, G.W., Sheu, C.-J., and von Halle, E.: Mutat. Res. 76, 191 (1980).

169. Aeschbacher, H.U., Milon, H., and Wurzner, H.P.: Mutat. Res. 57, 193 (1978).

170. Degraeve, N.: Mutat. Res. 55, 31 (1978).

171. International Agency for Research on Cancer: IARC Monogr. 26, 349 (1981).

172. International Agency for Research on Cancer: IARC Monogr. Suppl. 4, 222, 257 (1982).

173. Savkovic, N., Pecevski, J., Radivojevic, D., Vuksanovic, L., and Djuricic, E.: Mutat. Res. 80, 299 (1981).

174. Veckova, L., Vondreys, V., and Necasek, J.: Folia Microbiologica (Prague) 15, 76 (1970).

175. Kihlman, B.A. (ed.): "Caffeine as an Environmental Mutagen and the Problem of Synergistic Effects." Special issue of Mutation Research, Elsevier, Amsterdam, Vol. 26, No. 2, 1974, 155 pp.

176. Thayer, P.S., and Palm, P.E.: CRC Crit. Rev. Toxicol. 3, 345 (1975).

177. Lockhart, M.L., and Shankel, D.M.: Mol. Gen. Genet. 179, 253 (1980).

178. Wun, K.L., and Shafer, R.H.: Radiat. Res. 90, 310 (1982).

179. Tohda, H., and Oikawa, A.: Mutat. Res. 107, 387 (1983).

180. Panigrahi, G.B., and Rao, A.R.: Mutat. Res. 122, 347 (1983).

181. Christie, N.T., Drake, S., Meyn, R.E., and Nelson, J.A.: Cancer Res. 44, 3665 (1984).

182. Hansson, K., Kihlman, B.A., Tanzarella, C., and Palitti, F.: Mutat. Res. 126, 251 (1984).

183. Balachandran, R., and Srinivasan, A.: Carcinogenesis 3, 151 (1982).

184. Weinstein, D., Mauer, I., Katz, M.L., and Kazmer, S.: Mutat. Res. 31, 57 (1975).

185. Goldman, A.S., and Yakovac, W.C.: Proc. Soc. Exp. Biol. Med. 118, 857 (1965).

186. Kanoh, S., and Moriyama, I.S.: Teratology 16, 110 (1977).

187. Bucnick, I.S., Leikin, S., and Hoeck, L.E.: Am. J. Dis. Child. 90, 286 (1955).

188. Sobel, D.E.: AMA Arch. Gen. Psychiatry 2, 606 (1960).

189. Connors, T.A.: Cytotoxic Agents in Teratogenic Research. In "Teratology, Trends and Applications" (C.L. Berry and D.E. Poswillo, eds.), Springer-Verlag, New York, 1975, p. 49.

190. Joneja, M.G., and LeLiever, W.C.: Toxicol. Appl. Pharmacol. 27, 408 (1974).

191. DeMyer, W.: Neurology 14, 806 (1964).

192. Cohlan, S.Q., and Kitay, D.: J. Pediat. 66, 541 (1965).

193. Ferm, V.H.: Science 141, 426 (1963).

194. Courtney, K.D., and Valerio, D.A.: Teratology 1, 163 (1968).

195. Joneja, M., and Ungthavorn, S.: Teratology 2, 235 (1969).

196. DeMyer, W.: Arch. Anat. 48, 181 (1965).

197. Lapointe, G., and Nosal, G.: Biol. Neonate 36, 273 (1979).

198. Robinson, G.C., Bromitt, J.R., and Miller, J.R.: Pediatrics 32, 115 (1963).

199. Tanimura, T.: Effects on Macaque Embryos of Drugs Reported or Suspected to be Teratogenic to Humans. In "The Use of Non-Human Primates in Research on Human Reproduction" (E. Diczfalusy and C.C. Standley, eds.), WHO Research and Training Center on Human Reproduction, Stockholm, Sweden, 1972, p. 293.

200. Sheveleva, G.A., Kiriushchenkov, A.P., Sheina, N.I., and Silant'eva, I.V.: Farmakol. Toksikol. (Russian) 47, 78 (1984).

201. Nishimura, H., and Nakai, K.: Science 127, 877 (1958).

202. Ritter, E.J., Scott, W.J., Wilson, J.G., Mathionos, P.R., and Randall, J.L.: Teratology 25, 95 (1982).

203. Ritter, E.J.: Fund. Appl. Toxicol. 4, 352 (1984).

204. Elmazar, M.M., McElhatton, P.R., and Sullivan, F.M.: Human Toxicology 1, 53 (1981).

205. Elmazar, M.M., McElhatton, P.R., and Sullivan, F.M.: Toxicology 23, 57 (1982).

206. Collins, T.F., Welsh, J.J, Black, T.N., and Ruggles, D.I.: Food Chem. Toxicol. 21, 763 (1983).

207. Young, J.F., and Kimmel, C.A.: Toxicologist 3, 65 (1983).

208. Fujii, T., and Nakatsuka, T.: Teratology 28, 29 (1983).

209. Skalko, R.G., Poche, P.D., and Kwasigroch, T.E.: Toxicology 14, 7 (1984).

210. Arnaud, M.J., Bracco, I., Sauvageat, J.L., and Clerc, M.F.: Toxicol. Lett. 16, 271 (1983).

211. Knutti, R., Rothweiler, H., and Schlatter, C.: Arch. Toxicol. (Suppl.) 5, 187 (1982).

212. Weathersbee, P.S., Olsen, L.K., and Lodge, J.R.: Postgrad. Med. 62, 64 (1977).

213. Kurppa, K., Holmberg, P.C., Kuosma, E., and Sax'en, L.: Am. J. Public Health. 73, 1397 (1983).

214. Keller, S.J., and Smith, M.K.: Teratogen., Carcinogen. Mutagen. 2, 261 (1982).

215. Braun, A.G., Buckner, C.A., Emerson, D.J., and Nichinson, B.B.: Proc. Nat. Acad. Sci. (U.S.A.) 79, 2056 (1982).

216. Bournias-Vardiabasis, N., Teplitz, R.L., Chernoff, G.F., and Seecof, R.L.: Teratology 28, 109 (1983).

217. Tuchmann-Duplessis, H., and Mercier-Parot, L.: C.R. Acad. Sci. (Paris) 254, 1535 (1962).

218. Tatamatsu, M., Takahashi, M., Tsuda, H., Ogiso, T., and Ito, N.: Toxicol. Lett. 1, 201 (1978).

219. Lacassagne, A., and Duplan, J.-F.: C.R. Acad. Sci. (Paris) 249, 810 (1959).

220. Hakim, S.A.E.: Nature (London) 189, 198 (1962).

221. Hakim, S.A.E.: Indian J. Cancer 5, 183 (1968).

222. Truhaut, R., and DeClercq, M.: C.R. Acad. Sci. (Paris) 253, 1506 (1961).

223. Toth, B.: Anticancer Res. 2, 71 (1982).

224. NCI: "Bioassay of Emetine for Possible Carcinogenicity," NCI Carcinog. Tech. Rep. Ser. No. 43, DHEW Publ. No. (NIH) 78-843. National Cancer Institute, Bethesda, Maryland, 1978.

225. Johansson, S.L.: Int. J. Cancer 127, 521 (1981).

226. Mohr, U., Althoff, J., Ketkar, M.B., Conradt, P., and Morgareidge, K.: Food Chem. Toxicol. 22, 377 (1984).

227. Takayama, S., and Kuwabara, N.: Gann 73, 365 (1982).

228. Yamagami, T., Handa, H., Takeuchi, J., Munemitsu, H., Aoki, M., and Kato, Y.: Surg. Neurol. 20, 323 (1983).

229. Macklin, A.W., and Szot, R.J.: Drug Chem. Toxicol. 3, 135 (1980).

230. Flaks, B.: Pathol. Res. Pract. 163, 373 (1978).

231. Boyland, E., Charles, R.T., and Gowing, N.F.C.: Br. J. Cancer 15, 252 (1961).

232. Boyland, E., Busby, E.R., Dukes, C.E., Grover, P.L., and Manson, D.: Br. J. Cancer 18, 575 (1964).

233. Schmahl, D., and Osswald, H.: Arznemittel.-Forsch. 20, 1461 (1970).

234. Schmahl, D.: Recent Results Cancer Res. 52, 18 (1975).

235. Weisburger, E.K.: Cancer 40, 1935 (1977).

236. Truhaut, R., DeClercq, M., Loisillier, F., and Colin, S.: Path. Biol. 12, 39 (1964).

237. Goldberg, L. (ed.): Studies on Nicotine and Cotinine. In: Food Cosmet. Toxicol. 4, 110 (1966).

238. Schmahl, D., and Osswald, H.: Z. Krebsforsch. 71, 198 (1968).

239. Schutte, K.H.: J. Natl. Cancer Inst. 41, 821 (1968).

240. Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K., and Gori, G.B.: Cancer Res. 33, 3069 (1973).

241. Zeitlin, B.R.: Lancet 1, 1066 (1972).

242. Bauer, A.R. Jr., Rank, R.K., Kerr, R., Straley, R.L., and Mason, J.D.: Life Sci. 21, 63 (1977).

243. Wurzner, H.-P., Lindstrom, E., Vuataz, L., and Luginbuhl, H.: Food Cosmet. Toxicol. 15, 289 (1977).

244. Palm, P.E., Arnold, E.P., Nick, M.S., Valentine, J.R., and Doerfler, T.E.: Toxicol. Appl. Pharmacol. 74, 364 (1984).

245. Hirakawa, T., Tanaka, M., and Takayama, S.: Toxicol. Lett. 3, 55 (1979).

246. Welsch, C., and Meites, J.: Experientia 26, 1133 (1970).

247. Verdeal, K., Erturk, E., and Rose, D.P.: Eur. J. Cancer Clin. Oncol. 19, 825 (1983).

248. Lacassagne, A., Buu-Hoi, N.P., Giao, N.B., and Ferando, R.: Bull. Cancer 55, 87 (1968).

249. Lupulescu, A.: J. Natl. Cancer Inst. 71, 1077 (1983).

250. Bock, F.G.: Banbury Rep. Ser. 3, 129 (1980).

251. Gurkalo, V.K., and Volfson, N.I.: Arch. Geschwulstforsch. 52, 259 (1982).

252. Welsch, C.W., Brown, C.K., Goodrich-Smith, M., Chiusano, J., and Moon, R.C.: Cancer Res. 40, 3095 (1980).

253. Armuth, V, and Berenblum, I.: Carcinogenesis 2, 977 (1981).

254. Nomura, T.: Cancer Res. 40, 1332 (1980).

255. Govindwar, S.P., Kachole, M.S., and Pawar, S.S.: Food Chem. Toxicol. 22, 371 (1984).

256. Ranadive, K.J., Gothoskar, S.V., and Tezabwala, B.U.: Int. J. Cancer 10, 652 (1972).

257. Fujita, J., Tokuda, H., Sugawara, K., and Yoshida, O.: Toxicol. Lett. 22, 261 (1984).

258. Glazko, G.D., Dill, W.A., Wolf, L.M., and Kazenko, A.: J. Pharmacol. Exp. Ther. 118, 377 (1956).

259. Numerof, P., Gordon, M., and Kelly, J.M.: J. Pharmacol. Exp. Ther. 115, 427 (1955).

260. Clemens, J.A., Shaar, C.J., Smastig, E.B., and Matsumoto, C.: Horm. Metab. Res. 6, 187 (1974).

261. Cazenave, J.-P., Reimers, H.-J., Perey, D.Y.E., and Mustard, J.F.: Lancet 2, 1571 (1974).

262. Lacassagne, A., Buu-Hoi, N.P., and Ba-Giao, N.: C.R. Acad. Sci. (Paris) 270, 746 (1970).

263. Faddejeva, M.D., Belyaeva, T.N., Novikov, J.P., and Shalabi, H.G.: IRCS Med. Sci. Cancer 8, 612 (1980).

264. Naiti, M., Nandi, R., and Chaudhuri, K.: FEBS Lett. 142, 280 (1982).

265. Szuts, T., Olsson, S., Lindquist, N.G., and Ullberg, S.: Toxicology 10, 207 (1978).

266. Stehlik, G., Kainzbauer, J., Tausch, H., and Richter, O.: J. Chromatogr. 232, 295 (1982).

267. Oppelt, W.W., Zange, M., Ross, W.E., and Remmer, H.: Xenobiotica 5, 539 (1975).

268. Turner, D.M., Armitage, A.K., Briant, R.H., and Dollery, C.T.: Xenobiotica 5, 539 (1975).

269. Litterst, C.L., Mimnaugh, E.G., Reagan, R.L., and Gram, T.E.: Drug Metab. Dispos. 3, 259 (1975).

270. Hill, P., and Wynder, E.L.: Cancer Lett. 6, 251 (1979).

271. Nguyen, T.L., Gruenke, L.D., and Castagnoli, N. Jr.: J. Med. Chem. 22, 259 (1979).

272. Sullivan, H.R., Billings, R.E., Occolowitz, J.L., Boaz, H.E., Marshall, F.J., and McMahon, R.F.: J. Med. Chem. 13, 904 (1970).

273. Tank-Liu, D.D., Williams, R.L., and Riegelman, S.: J. Pharmacol. Exp. Ther. 224, 180 (1983).

274. Callahan, M.M., Robertson, R.S., Arnaud, M.J., Branfman, A.R., and McComish, M.F.: Drug Metab. Dispos. 10, 417 (1982).

275. Tank, B.K., Grant, D.M., and Kalow, W.: Drug Metab, Dispos. 11, 218 (1983).

276. Ferrero, J.L., and Neims, A.H.: Life Sci. 33, 1173 (1983).

277. Arnaud, M.J.: Biochem. Med. 16, 67 (1976).

278. Szczawinska, K., Ginelli, E., Bartosek, I., Gambazza, C., and Pantarotto, C.: Chem.-Biol. Interact. 34, 345 (1981).

279. Castle, M.C., and Mead, J.A.R.: Biochem. Pharmacol. 27, 37 (1978).

280. Owellen, R.J., Hartke, C.A., and Hains, F.O.: Cancer Res. 37, 2597 (1977).

281. Bender, R.A., Castle, M.C., Margileth, D.A., and Oliverio, V.T.: Clin. Pharmacol. Ther. 22, 430 (1977).

282. Creasey, W.A., and Marsh, J.C.: Proc. Am. Assoc. Cancer Res. 14, 57 (1973).

283. Labarthe, D.R., and O'Fallon, W.M.: J. Am. Med. Assoc. 243, 2304 (1980).

284. Ross, R.K., Paganini-Hill, A., Krailo, M.D., Gerkins, V.R., Henderson, B.E., and Pike, M.C.: Cancer Res. 44, 3106 (1984).

285. Newball, H.H., and Byar, D.P.: Urology 2, 525 (1973).

286. Weisburger, J.H., Cohen, L.A., and Wynder, E.L.: On the Etiology and Metabolic Epidemiology of the Main Human Cancers. In "Origins of Human Cancer" (H.H. Hiatt, J.D. Watson, and J.A. Winsten, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1977, p. 567.

287. Miller, A.B.: Coffee and Cancer. In "Carcinogens and Mutagens in the Environment" (H.F. Stich, ed.), Vol. III, CRC Press, Boca Raton, Florida, 1983, p. 14.

288. Stocks, P.: Br. J. Cancer 24, 215 (1970).

289. Heyden, S.: Z. Ernahrungswiss. 14, 11 (1972).

290. Cole, P.: Lancet 1, 1335 (1971).

291. Simon, D., Yen, S., and Cole, P.: J. Natl. Cancer Inst. 54, 587 (1975).

292. Morgan, R.W., and Jain, M.G.: Can. Med. Assoc. J. 111, 1067 (1974).

293. Shennan, D.H.: Br. J. Cancer 28, 473 (1973).

294. Wynder, E.L., Mabuchi, K., and Whitemore, W.F.: J. Natl. Cancer Inst. 53, 1619 (1974).

295. Armstrong, B., Garrod, A., and Doll, R.: Br. J. Cancer 33, 127 (1976).

296. MacMahon, B., Yen, S., Trichopoulos, D., Warren, K., and Nardi, G.: New Engl. J. Med. 304, 630 (1981).

297. Svoboda, G.H.: Kirk-Othmer Encycl. Chem. Technol. (2nd ed) 1, 758 (1963).

SOURCE BOOKS AND MAJOR REVIEWS FOR SECTION 5.3.2.3

1. Bull, L.B., Culvenor, C.C.J., and Dick, A.T.: "The Pyrrolizidine Alkaloids." John Wiley and Sons, New York, 1968, 293 pp.

2. McLean, E.K.: Pharmacological Reviews 22, 429-483 (1970).

3. International Agency for Research on Cancer: "Some Naturally Occurring Substances," IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 10. Int. Agency Res. Cancer, Lyon, France, 1976, pp. 265-342.

4. International Agency for Research on Cancer: "Some Pharmaceutical Drugs," IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 24. Int. Agency Res. Cancer, Lyon, France, 1980, pp. 211-241.

5. International Agency for Research on Cancer: "Some Antineoplastic and Immunosuppressive Agents," IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 26. Int. Agency Res. Cancer, Lyon, France, 1981, pp. 349-384.

6. Schoental, R.: Carcinogens in Plants and Microorganisms. In "Chemical Carcinogens" (C.E. Searle, ed.), ACS Monograph 173, American Chemical Society, Washington, D.C., 1976, pp. 626-689.

7. Culvenor, C.C.J., and Jago, M.V.: Carcinogenic Plant Products and DNA. In "Chemical Carcinogens and DNA" (P.L. Grover, ed.), CRC Press, Boca Raton, Florida, 1979, pp. 161-186.

8. Miller, A.B.: Coffee and Cancer. In "Carcinogens and Mutagens in the Environment" (H.F. Stich, ed.), Vol. III, CRC Press, Boca Raton, Florida, 1983, pp. 13-20.

9. Timson, J.: Mutat. Res. 47, 1-52 (1977).

10. Thayer, P.S., and Palm, P.E.: CRC Crit. Rev. Toxicol. 3, 345-369 (1975).

11. Oser, B.L., and Ford, R.A.: Drug Chem. Toxicol. 4, 311-329 (1981).

12. Hakim, S.A.E.: Maharashtra Med. J. 17, 109-130 (1970).