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INDANONE DERIVATIVES AND RELATED COMPOUNDS: BRACKEN FERN TOXINS

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

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5.3.2.1 Bracken Fern Toxins

5.3.2.1.1 Introduction

Bracken fern (Pteridium aquilinum), a primitive plant belonging to the Family Polypodiaceae (Order Pteridophyta), occurs worldwide and is cultivated in Japan, New Zealand, Canada and in the northeastern United States for human consumption as a vegetable or in salads. Yet, the plant contains toxins and has long been known to cause characteristic illness in grazing animals. It first came to the attention of oncologists when an association was noted between chronic ingestion of the plant and the emergence of urinary bladder and intestinal tumors in cattle and sheep. Carcinogenesis bioassays using laboratory animals demonstrated that bracken fern not only induces tumors of the urinary bladder and the intestine, but also neoplasms of other sites in several species. Recent epidemiological studies have shown that human consumption of bracken fern is correlated with an increased incidence of esophageal carcinomas in Japan.

Much time and effort have been expended in the identification and characterization of bracken toxins by various investigators around the world. However, the exact chemical identity of the components responsible for the toxic and carcinogenic properties of bracken fern remains elusive. No single chemical isolated so far can mimic all aspects of the toxicity and carcinogenicity displayed by intact bracken fern, when administered to susceptible animal species. The toxins identified in bracken fern, suspected and/or tested for carcinogenicity, include shikimic acid (1), pterolactam (2), pterosins and

pterosides (3-5), ptaquiloside (aquilide A) (6-8), glycosides of quercetin (rutin and isoquercitrin) and of kaempferol (astragalin and tilroside) (9, 10) and tannin (11). The structural formulas of some of these toxins are shown in Table XXXIII.

For many years, the chemical, biological and carcinogenic properties of bracken fern have been the subjects of extensive research by several investigators, particularly I.A. Evans in the United Kingdom, I. Hirono in Japan and A.M. Pamukcu in the United States. Many of their important findings are covered in several reviews (e.g., 12-14).

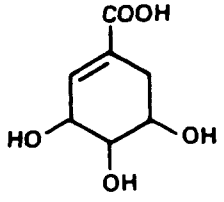
5.3.2.1.2 Physicochemical Properties and Biological Effects

5.3.2.1.2.1 PHYSICAL AND CHEMICAL PROPERTIES

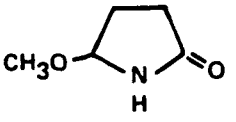
There appears to be a consensus that the chemical constituents in bracken fern which are carcinogenic are also the agents which confer to the plant its toxicity. The compounds are heat stable and methanol-, ethanol-, chloroform- and water-soluble. Some physicochemical properties of shikimic acid, pterolactam, pterosin B, pteroside B and ptaquiloside (aquilide A) are presented in Table XXXIV.

Shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) is a water-soluble, white solid. It is not stable in solution and may undergo spontaneous aromatization and oxidation. Shikimic acid, found widespread in plants, is an intermediate in the biosynthesis of many aromatic plant constituents from carbohydrates. The chemistry, biochemistry and distribution of this naturally occurring substance have been extensively discussed in a review in 1965 (15).

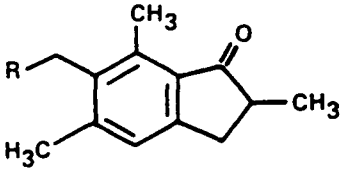
Pterolactam (5-methoxy-2-pyrrolidone) is a novel compound isolated from



Shikimic acid

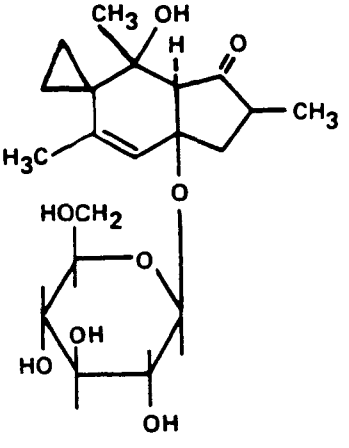


Pterolactam



Pterosin B
(R = — CH_2OH)

Pteroside B
(R = — CH_2O — glucose)



Ptaquiloside
(Aquilide A)

Table XXXIII

Some Bracken Fern Toxins Which Have Been Tested for Carcinogenic Activity

Table XXXIV
Physicochemical Properties of Some Bracken Fern Toxins[a]

Compound[b]	Empirical formula	m.p. (°C)	Specific rotation	IR ν_{max}^{KBr} (cm^{-1})
Shikimic acid	$C_7H_{10}O_5$	191-192	$[\alpha]_D^{18} = -185°$ (MeOH)	--
Pterolactam	$C_5H_9O_2N$	56-67	$[\alpha]_D^{25} = +2.0°$ ($CHCl_3$)	1,700
Pterosin B	$C_{14}H_{18}O_2$	109-110	$[\alpha]_D^{25} = -31.9°$ (MeOH)	3,300, 1,705, 1,670
Pteroside B	$C_{20}H_{28}O_7$	120-122	$[\alpha]_D^{25} = -48.8°$ (MeOH)	3,360, 1,683, 1,605
Ptaquiloside (Aquilide A)	$C_{20}H_{30}O_8$	173-174[c]	$[\alpha]_D^{22} = -188.0°$ (MeOH)	3,400, 1,724, 1,640

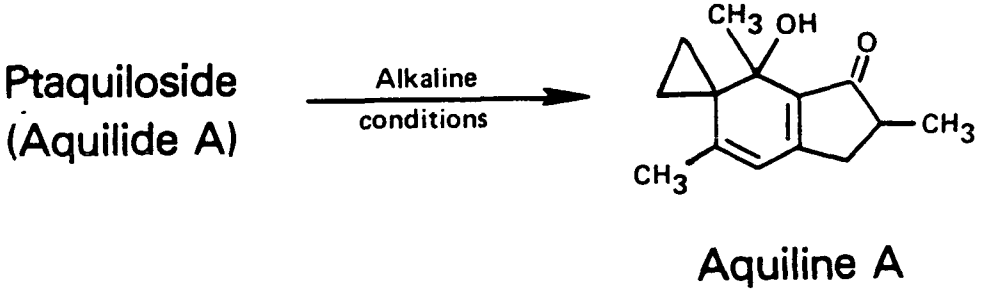
[a]Data summarized from Stavric, B., and Stoltz, D.R. [Food Cosmet. Toxicol. 14, 141-145 (1976)]; Takatori, K., Nakano, S., Nagata, S., Okumura, K., Hirono, I., and Shimizu, M. [Chem. Pharm. Bull. 20, 1087 (1972)]; Fukuoka, M., Kuroyanagi, M., Yoshihira, K., and Natori, S. [Chem. Pharm. Bull. 26. 2365-2385 (1978)]; and Niwa, H., Ojika, M., Wakamatsu, K., Yamada, K., Hirono, I., and Matsushita, K. [Tetrahedron Lett. 24, 4117-4120 (1983)].

[b]See Table XXXIII for structural formulas.

[c]Ptaquiloside tetraacetate.

bracken fern by Takatori et al. (2) in 1972. It was extracted by methanol from young leaves of the plant. It forms colorless leaflets when recrystallized from petroleum ether.

Pterosin B and pteroside B are among more than thirty 1-indanone derivatives isolated from the leaves and rhizomes of bracken fern (16). The characterization and absorption spectra of these compounds have been extensively described (3-5). Ptaquiloside (aquilide A), another characteristic constituent of bracken fern, is a norsesquiterpene glucoside of the illudane type. The compound is rather unstable and readily converts to pterosins under acidic conditions. The half-life of ptaquiloside in 0.01 M sulfuric acid-methanol at 22°C is about 2 hours. Under alkaline conditions, the glucose moiety and the proton at position 9 are removed, forming an aglycon (designated aquiline A) with an unsaturated bond between C-4 and C-9 (6, 8):



Ptaquiloside is a possible biosynthetic precursor of pterosins and pterosides. Its concentration in freeze-dried bracken fern is about 2.5 g/kg (8).

Quercetin and kaempferol are flavonoids occurring in conjugated or unconjugated forms in many plants. Tannin is a generic term referring to a group of phenolic compounds widely distributed in the plant kindgom as heterogenous polymeric substances. In bracken fern, the concentrations of quercetin, kaempferol (as liberated aglycones) and tannin, in terms of g/kg dry weight, are 0.57-0.86, 1.10-2.55 and 2.45, respectively (14). These compounds are further discussed in Section 5.3.2.6.2 and Section 5.3.2.6.3.

5.3.2.1.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENICITY

Toxic effects. Poisoning of cattle as a result of excessive feeding on bracken fern was first scientifically recorded in 1893 (17, 18). Since then, the toxic action of bracken fern on various animal species has been repeatedly established in the field as well as demonstrated in experimental studies. When ingested by horses, pigs, sheep, rats and pigeons, bracken fern produces the typical nervous lesions of avitaminosis B_1. The affected animals show anorexia, staggering and incoordination. This syndrome is caused by the presence of thiaminase in the plant and can be reversed by the administration of thiamine.

Ingestion of bracken fern by cattle and several other animal species results in "cattle bracken poisoning," characterized by bone marrow aplasia, damage to the gastrointestinal mucosa and generalized hemorrhage (19). Continued feeding of the plant leads to "chronic enzootic bovine hematuria," resulting from characteristic lesions produced in the urinary bladder mucosa (20). These manifestations of bracken fern toxins in cattle do not differ significantly from those caused by ionizing radiation or radiomimetic chemicals and they cannot be prevented or remedied by supplementation with thiamine (12, 21).

It is possible that bracken fern contains more than one radiomimetic principles. The acute bracken fern toxicity in cattle can be reproduced with ptaquiloside (22) but not with bracken fern extracts containing pterosins and pterosides (23). These findings led Hirono et al. (22) to suggest that the causative factor in "cattle bracken poisoning" is ptaquiloside.

The shikimic acid present in bracken fern possibly accounts for the plant's acute toxicity in cattle. The identity of shikimic acid in bracken fern has been established by Evans and Osman (1). The compound is present at a much higher concentration in the rhizomes than in the frond (24-26). Shikimic acid is mutagenic, carcinogenic and lethal to mice on i.p. injection (27), but there is little or no information on the cytologically established radiomimetic effects of the compound. The LD_{50} of shikimic acid in mice by i.p. injection is 1 g/kg (1).

Mutagenic effects. Aqueous and organic solvent extracts of bracken fern contain substances mutagenic to the Salmonella typhimurium strains TA100 and TA98 after microsomal activation (7, 28). Milk from cows fed bracken fern and urine from rats ingesting bracken fern reversed S. typhimurium TA100 and TA98 mutants, without the addition of S-9 mix (29, 30). Bracken fern extracts also exhibit positive mutagenic responses in the Drosophila test (27, 31) and in the mouse dominant lethal assay (32). They induce sister-chromatid exchange in V79 Chinese hamster cells (7) and cause damage to DNA in gastrointestinal epithelial cells in vitro (cited in ref. 33). The mutagenicity is substantially reduced in processed bracken fern (used as a human food) after treatment with salt, sodium bicarbonate or wood ash (28).

Besides flavonoids and their glycosides (discussed in Section 5.3.2.6.3), other mutagens are believed to be present in the extracts and fractions of

bracken fern (28, 30). Evans and Osman (1) suggested that shikimic acid may be the mutagenic principal in bracken fern extracts, on the basis of the finding that shikimic acid induces dominant lethal mutations in mice. However, neither shikimic acid nor its bacterial and mammalian metabolites (see Fig. 9 for structural formulas) showed any activity in the Ames test with or without metabolic activation (34-36). Furthermore, shikimic acid did not produce chromosomal aberrations in Chinese hamster cells in culture (37) and its dominant lethal effects on mice were not reproducible (38). More than twenty pterosins, pterosides and their derivatives isolated from fronds of bracken fern have been examined for mutagenicity using S. typhimurium and mammalian cells (FM3A cells). None of the compounds exhibited mutagenicity in these systems or caused chromosomal aberrations in Chinese hamster cells (16). Van der Hoeven et al. (8) have recently isolated from bracken fern a potent mutagenic compound, designated aquilide A (also called ptaquiloside), which is responsible for more than 50% of the mutagenic activity observed in a methanol extract of the plant. Aquilide A is strongly mutagenic to S. typhimurium strains TA100 and TA98 under alkaline conditions and is a potent inducer of sister-chromatid exchange and HGPRT-deficient mutants in V79 chinese hamster cells, and of unscheduled DNA synthesis in human fibroblast (8).

Reproductive and teratogenic effects. Studies with mice, quail and Drosophila have indicated that bracken fern has a sterilizing effect on male animals (21, 27). Pregnant mice fed a diet containing 33% bracken fern showed maternal weight loss, intrauterine growth suppression (39) and an increase in abortion rate (40). Significant abnormalities in the ribs and the sternebrae with retarded ossification were found in the fetuses (40). In another experiment, however, when given to mice at 0.25 or 1.00 g/kg/day through day 18 of pregnancy, shikimic acid exhibited no teratogenic effects in the newborn (41).

5.3.2.1.3 Carcinogenicity and Structure-Activity Relationships

5.2.3.1.3.1 OVERVIEW

In advanced cases of "chronic enzootic bovine hematuria," the development of neoplastic lesions of various forms and sizes in the urinary bladder is a common finding. In 1965, Rosenberger and Heeschen (42) first described changes of a polypous-tumorous nature in the bladder mucosa of five cows with hematuria caused by bracken fern. Subsequent studies by other investigators substantiated the carcinogenicity of bracken fern in the urinary bladder of cows and, in addition, demonstrated that bracken fern contain toxins which are potent carcinogens toward many sites in various animal species. Dried and fresh plant, or solvent extract of bracken fern, induce bladder cancer in cows, guinea pigs, rats and mice, intestinal tumors in sheep, hamsters, guinea pigs, Japanese quails, toads, rats and mice, lymphatic leukemia and lung tumors in mice, mammary gland tumors in rats and hepatomas in toads (Table XXXV). Solvent extracts of urine or milk from cattle fed bracken fern also induced tumors of the bladder in calves, dogs, rats and mice (29, 67, 68).

Comparative studies have shown that the latent period for the induction of intestinal tumors in rats is shorter with the immature young fern than with the mature fern (50). All parts of the plant are oncogenic; however, the carcinogenicity of the rhizomes is greater than that of the fronds, which in turn has a greater carcinogenic activity than the stalks (60). Weak but definite carcinogenic activity still remains in processed bracken fern treated with boiling water containing sodium bicarbonate, sodium chloride or wood ash (59).

There is some evidence that shikimic acid (1), tannin (11), quercetin and kaempferol (69) isolated from bracken fern display carcinogenic activity (see also Section 5.3.2.6.2 and Section 5.3.2.6.3). However other substance(s),

Table XXXV
Carcinogenicity of Bracken Fern by Oral Administration[a]

Species and strain	Principal organs affected	Reference
Cow, Turkish	Urinary bladder	(43-45)
Sheep (Swaledale x Scottish Blackface)	Intestine	(21)
Hamsters, Syrian golden	Intestine	(13, 21)
Guinea pig, --	Intestine, urinary bladder	(21, 46, 47)
Quail, Japanese (Coturnix coturnix japonica)	Intestine	(21, 46)
Toad, Egyptian (Bufo regularis)	Intestine, liver	(48)
Mouse, Swiss	Lung, liver, intestine, haematopoietic tissues	(21, 32, 49)
Mouse, C57BL/6	Intestine	(50)
Mouse, dd	Lung	(50)
Mouse, ICR	Urinary bladder[b]	(51)
Mouse, Swiss	Urinary bladder[c]	(52)
Rat, Glaxo	Intestine	(53)
Rat, Albino	Intestine, urinary bladder	(45, 54-57)
Rat, ACI	Intestine, urinary bladder	(58-63)
Rat, Wistar	Intestine	(16, 64)
Rat, Fischer	Intestine, urinary bladder	(16, 65)
Rat, Sprague-Dawley	Intestine, urinary bladder, mammary gland	(65)
Rat, Sprague-Dawley (CD)	Mammary gland	(66)

[a]Dried or fresh bracken fern, or solvent extracts of bracken fern, were mixed in the diet of tested animals, unless otherwise indicated.

[b]Animals were fed powdered bracken fern mixed with a basic diet after the implantation of a glass bead into the urinary bladder.

[c]Pellets composed of bracken fern extract and cholesterol were surgically implanted into the urinary bladder of the animals.

more potently carcinogenic than these compounds, is/are believed to be present in the plant. Pterolactam, pterosin B, pteroside B and extracts of bracken fern containing other 1-indanone derivatives, were found to be not carcinogenic in rats and mice (16, 50, 70). In 1984, Hirono and coworkers (71, 71) isolated from bracken fern a novel norsequiterpene glucoside of the illudane type, called ptaquiloside (aquilide A) (see Table XXXIII for structure), and claimed that ptaquiloside may be the substance responsible for the hematotoxic, mutagenic and carcinogenic effects of bracken fern. The carcinogenicity studies of some bracken fern toxins are summarized in Table XXXVI.

5.3.2.1.3.2 CARCINOGENICITY OF BRACKEN FERN

Demonstration that ingested bracken fern is the etiological agent of bovine bladder cancer in several regions of the world has been provided by experimental testings in cows as well as in other animals. In a study in which 18 cows ranging from 1.5 to 4 years of age and weighing from 100 to 150 kg were fed dried (300-600 gm) or fresh (400-1,000 gm) bracken fern daily, 10 surviving animals which received bracken fern at the lower dietary levels, developed urinary bladder carcinomas, papillomas and hemangiomas after a mean feeding period of 550 days (43); these animals also had hematuria. Similar findings were obtained from prolonged, low-level feeding of bracken fern to calves; the induced neoplasms were histologically indistinguishable from the naturally occurring bovine bladder tumors (44, 45).

The bladder carcinogenicity of the urine and milk from cows fed bracken fern has been assessed by the pellet implantation technique (see Section 4.3.3.5, Vol. I). Urine or milk from these animals was extracted with organic solvents and the extract mixed with cholesterol for the preparation of the pellets. Pellets containing these urine extracts introduced into the bladder

Table XXXVI
Carcinogenicity of Some Bracken Fern Toxins[a]

Compound	Species and strain	Route	Principal organs affected	References
Shikimic acid	Mouse, TF1	i.p. or i.g.	Stomach, haematopoietic tissue	(3)
	Rat, ACI	oral	None	(73)
Pterolactam	Mouse, Swiss	urinary bladder implantation	None	(50)
	Rat, ACI	oral	None	(50)
Pterosin B	Rat, Wistar	oral	None	(16, 70)
Pteroside B	Rat, Wistar	oral	None	(16, 70)
Ptaquiloside (Aquilide A)	Rat, Sprague-Dawley	oral	Mammary gland, intestine	(71, 72)

[a]See Table XXXIII for structural formulas.

induced carcinomas in mice (68) and hemangiomatous lesions in calves, dogs and rats (67). Pellets prepared with milk extracts caused bladder carcinomas in 10 of 24 mice; only 3 of 19 mice treated with control pellets (prepared from milk of cows fed a normal diet) bore these tumors (29). Further evidence that carcinogenic substance(s) is/are present in the milk of cattle consuming bracken fern stems from the findings that 9 of 34 rats fed whole milk and 11 of 56 rats fed freeze-dried powdered milk from cows ingesting bracken fern developed carcinomas of the urinary bladder, intestine or kidney (29).

When guinea pigs (21, 47) and rats (45, 54, 56, 57, 61) were given a basic diet supplemented with bracken fern or when rats (61, 66) were administered cold or hot water extracts of bracken fern as drinking water, significant incidences of urinary bladder neoplasms were found. Pamukcu et al. (52) induced urinary bladder carcinomas in Swiss albino female mice by implantation of pellets containing bracken fern into the bladder. Urinary bladder tumors were also induced in 4 of 15 ICR strain mice fed powdered bracken fern in a diet (25%) for 20 weeks, following surgical implantation of a glass bead into the bladder. No such tumors were observed in a group of mice fed the bracken fern diet without a glass bead in their bladder and in a control group bearing a glass bean implant and fed a normal diet (51).

In addition to bladder cancer, mice and rats exhibited tumors at other localizations following bracken fern administration. Lymphatic leukemia and pulmonary adenomas have been reported in Swiss mice fed a bracken fern diet (33%) every other week for a total period of 60 weeks (49). High incidence of lung tumors was also induced in Swiss mice (21) and in dd strain mice (50) fed bracken fern. Several studies have demonstrated that bracken fern induces a significant incidence of intestinal tumors in rats of various strains (see Table XXXV); spontaneous intestinal neoplasms are only seldom encountered in

rats. It appears that feeding of bracken fern to rats for short duration induces only intestinal tumors, whereas long-term feeding produces tumors of the intestine and the bladder simultaneously. No difference in intestinal tumor incidence was found between conventional and germ-free Wistar female rats, suggesting that microflora of the gut may not play an important role in bracken fern carcinogenesis (64). The carcinogenicity of processed bracken fern treated with boiling water containing wood ash, sodium bicarbonate or sodium chloride has been tested in ACI rats. Thus, the plant which has undergone processing for human food can still possess weak carcinogenic activity (59). Bracken fern powder which has been stored for 1 to 2 years induces intestinal tumors in ACI rats, although with a lower incidence and a longer latent period, suggesting that the carcinogenic activity of bracken fern is reduced by long-term storage (63). Recently, Hirono et al. (66) noted, in addition to ileal and urinary bladder tumors, a 87% incidence of mammary tumors in 15 female CD strain rats given a diet containing 30% bracken fronds for up to 260 days. Furthermore, these authors (74) observed bile duct proliferation and hyperplastic nodules in the liver in two strains of rats fed a diet containing bracken fern or water extract of the plant.

Several other animal species have been found to be susceptible to the carcinogenic action of bracken fern. Sheep and young hamsters fed bracken fern diet developed gastrointestinal neoplasms (13, 21). In the sheep, both intestinal and colonic adenocarcinomas were observed after feeding on bracken fern pellets for 8 months (21). In the hamster, adenocarcinomas were induced predominantly in the cecum and ileum (13, 21). When 34 Japanese quail (Coturnix coturnix japonica) received a methanol extract of dried bracken fern mixed with their normal diet for the first 5 months after hatching, 27 of the birds had adenocarcinomas of the intestine and colon which were not seen in

the controls (21, 46). The Egyptian toad (Bufo regularis) is an effective model for detecting the carcinogenicity of bracken fern. Seven ileal adenocarcinomas, 16 hepatomas and 6 tumors in the kidney due to metastases of the hepatomas were noted in 18 of 98 toads following forced feeding with bracken fern (10 mg/50 g body weight), once a week for only 20 weeks. Such lesions were not seen in 100 control toads (48).

5.3.2.1.3.3 CARCINOGENICITY OF BRACKEN FERN TOXINS

Shikimic acid. In 1971, Leach, Barber and Evans (27) reported the isolation of an active principle from bracken fern, carcinogenic to mice by i.p. injection. Chemical analysis showed that the isolated compound is shikimic acid (1). Following single i.p. injections (1-20 mg) or oral administration of 100 mg aqueous shikimic acid to 14 weanling TF1 strain mice, 6 glandular stomach tumors, 3 reticulum cell leukemias, 1 lymphocytic leukemia and 1 pulmonary adenoma were found in 9 animals. None of the 57 control mice displayed these tumors (1). Shikimic acid is also active in the BHK21 cell transformation assay (36). However, when shikimic acid was given to a group of ACI strain rats in the diet (0.1%) for 142 days, no tumors were induced (73). Furthermore, shikimic acid is the precursor in the biosynthesis of aromatic ring compounds in a wide variety of plants. Thus, shikimic acid is not considered to be the principal bracken fern carcinogen.

Pterolactam. The carcinogenic potential of pterolactam, a novel 5-membered lactam isolated from bracken fern, has been studied in mice and rats. No significant neoplasms were found in 30 female Swiss mice which received pellets containing pterolactam implanted surgically into the urinary bladder. The compound also failed to exhibit carcinogenic effects when fed or intragastrically administered to groups of young ACI strain rats (50).

A structurally-related chemical, caprolactam (2-oxohexamethyleneimine), used in the production of nylon-6, was also found noncarcinogenic in rats and mice under the study conditions of the U.S. National Toxicology Program (75).

Pterosin B and Pteroside B. These two major indanones are present in abundance in the fronds and rhizomes of bracken fern. Pterosin B, pteroside B or fractions of bracken fern extracts containing various other pterosins and pterosides were given to groups of Wistar rats in the diet for up to 205 days. The daily doses of pterosin B (4 mg) and pteroside B (10 mg) were each equivalent to about twice the level contained in a carcinogenic dose of dried bracken fern. Nonetheless, no neoplasms developed in the rats (16, 70). Phenindione (2-phenyl-1,3-indandione), an anticoagulant structurally related to pterosins, was also non-carcinogenic under similar study conditions (70).

Ptaquiloside (Aquilide A). Ptaquiloside (aquilide A) is structurally related to the illudins, a group of anti-cancer agents. This novel toxin was isolated from bracken fern independently by Niwa et al. (6) and by van der Hoeven and coworkers (7, 8) and was named by these two groups ptaquiloside and aquilide A, respectively. This compound is not only a potent genotoxic agent (see section on Mutagenic Effects), but it is also a strong carcinogen. In vitro transformation assays (8) have shown that ptaquiloside induces type III transformed foci in C3H $10T_{1/2}$ cells. When a fraction of the boiling water extract of bracken fern containing ptaquiloside was given to 7 female CD rats in the diet for 133 days, all animals developed mammary and intestinal tumors; in 5 of them, urinary bladder neoplasms were also found (71). Similar results were obtained in subsequent studies using purified ptaquiloside. High incidences of mammary adenomas (100% and 91%) and ileal adenocarcinomas (57% and 91%) were induced in two groups of female CD rats administered ptaquiloside (100-200 mg/kg body weight) once or twice a week for 8-9 weeks. Although

urinary bladder tumor was observed only in one rat, preneoplastic hyperplasia of the urinary bladder mucosa was present in 15 of 21 treated animals (71, 72). These are the findings which led to the conclusion that ptaquiloside represents the carcinogenic principle of bracken fern.

5.3.2.1.3.4 MODIFICATION OF CARCINOGENESIS

Hirono et al. (76) have reported that ingestion of bracken fern enhances the induction of tumors of the upper alimentary tract by N-propyl-N-nitroso-urethan in ACI rats. On the other hand, various chemicals have been shown to inhibit the carcinogenicity of bracken fern. For instance, administration of nicotinamide (0.5%) or phenothiazine (0.2%) in the diet decreases the tumorigenicity of bracken fern toward the intestine and urinary bladder by about 40-50% (55, 57). Similarly, a 25-30% reduction in the incidence of intestinal neoplasms induced by bracken fern was noted in rats fed a bracken fern-containing diet supplemented with disulfiram, calcium chloride or butylated hydroxyanisole; dietary calcium chloride or polyvinyl pyrrolidone supplementation suppressed the bracken fern-induced urinary bladder tumorigenesis by about 80% (56). However, a significantly higher incidence of urinary bladder carcinomas, however, was induced in rats fed a diet containing bracken fern and thiamine hydrochloride compared to rats fed a bracken fern-containing diet without thiamine (55).

5.3.2.1.4 Metabolism and Possible Mechanisms of Action

Among the chemicals isolated from bracken fern, shikimic acid, tannin, quercetin, kaempferol, pterolactam, pterosins, pterosides and ptaquiloside have been suspected to account for the carcinogenic action of the plant. However, the data available show that pterolactam, pterosins, and pterosides are not carcinogenic or mutagenic (see previous Sections). Tannin, quercetin,

ptaquiloside, and other phenolics and flavonoids present in bracken fern may play a role in the carcinogenic activity; the metabolisms and possible mechanisms of action of these substances are discussed in Sections 5.3.2.6.2 and 5.3.2.6.3.

The metabolism of shikimic acid has been studied quite extensively by Brewster, Jones and Parke (77-79). Figure 9 shows the major metabolic pathways of shikimic acid in the rat. After oral administration of shikimic acid to rats, benzoic acid, hippuric acid, 3,4,5,6-tetrahydrohippuric acid, hexahydrohippuric acid and cyclohexylcarbonyl-β-D-glucoronide were identified as principal metabolites in the urine (79). These metabolites are produced by an initial conversion of shikimic acid to cyclohexanecarboxylic acid by the intestinal flora followed by further metabolism in rat tissues (77, 78). Since none of the metabolites of shikimic acid exhibits any mutagenic and cell-transforming activity (36), the metabolism of shikimic acid probably represents a detoxification process. On the other hand, since shikimic acid itself is an α,β-unsaturated carboxylic acid, it may react with cellular nucleophiles (especially amino groups) by a Michael-type addition similarly to acrylic acid (see Appendix I).

The metabolism of ptaquiloside has not been investigated. However, on the basis of its chemical reactivity under acidic and alkaline conditions (see Section 5.3.2.1.2.1), it was speculated that ptaquiloside may be inactivated to pterosin B in the stomach, whereas in the intestine and in the urine of herbivorous animals some activation of ptaquiloside to aquiline A may occur and this results in intestinal and urinary bladder tumorigenesis (8). The findings that bracken fern-induced intestinal adenocarcinomas occur predominantly in the ileal region, where the pH level is the highest, appears to support this hypothesis. It also explains the observations that treatment

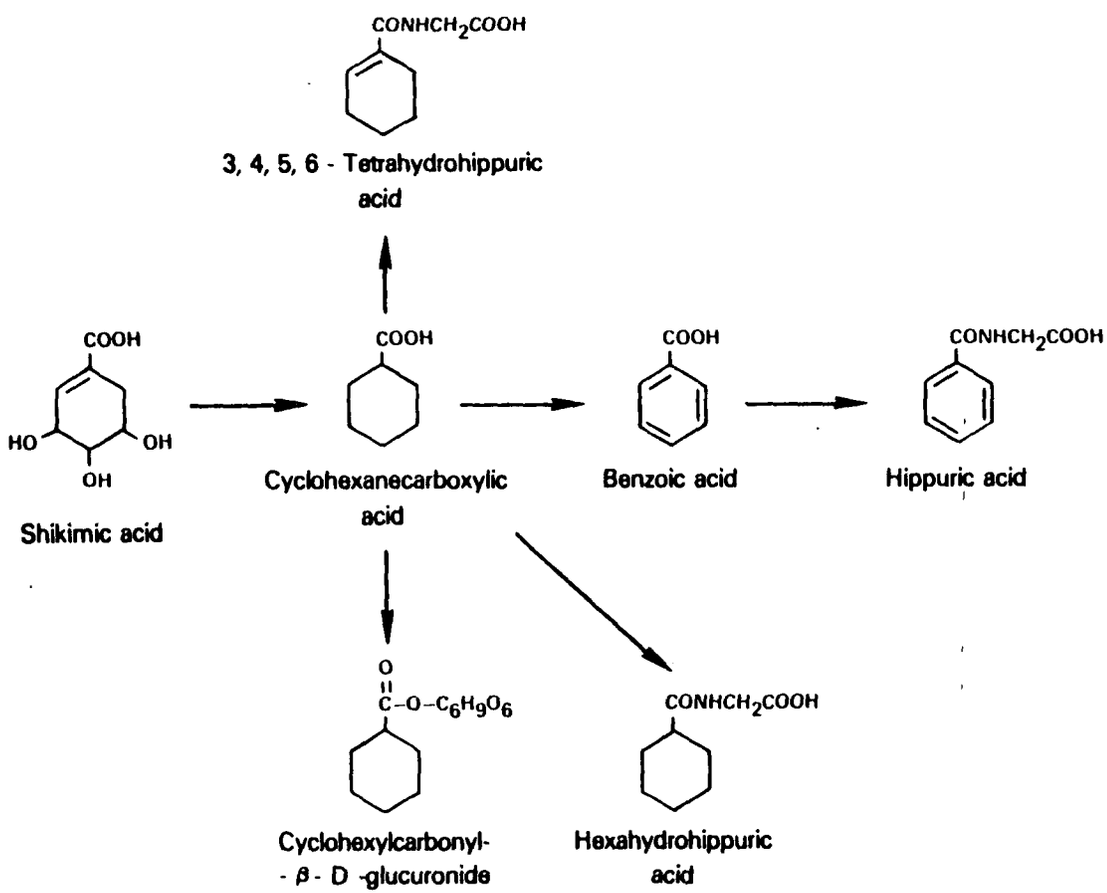


Fig. 9. Major metabolic pathways of shikimic acid in the rat.

with alkali reduces the carcinogenic activity of bracken fern (59) and that ptaquiloside is more potently mutagenic toward Salmonella under alkaline conditions (8). Aquiline A, the proposed reactive intermediate, may exert its carcinogenic and mutagenic action by virtue of the potential reactivity of the vinyl carbonyl and/or the cyclopropyl ring moieties in the molecule.

5.3.2.1.5 Environmental Significance

Bracken fern is an ubiquitous plant in the temperate and midtropic zones of the world. It grows well in moist, shady soils in a variety of habitats ranging from sea level to hilly pasture lands 2,000 feet or higher. As a forage contaminant, bracken fern has been responsible for many cases of cattle poisoning and death in various parts of the world, particularly in Scotland, Australia, New Zealand, Turkey and Brazil. A close association between the geographic distribution of bracken fern and the high rate of urinary bladder, intestinal and esophageal tumors in cattle and sheep in these countries has been observed (rev. in 13, 80).

In Japan, Canada and the northeastern United States, young fronds of bracken fern are consumed by humans as a food delicacy or salad greens. Moreover, indirect human exposure takes place through consumption of meat and milk (or dairy products) from animals grazing on bracken fern. For this reason, the marked regional prevalence of some forms of human cancer, such as esophageal and stomach cancers in Japan and North Wales, has been suspected to be linked to the exposure of bracken fern toxins. An epidemiological study of the cancer incidence of people living in a mountainous district of central Japan, where considerable quantities of bracken fern are consumed, revealed that daily intake of bracken fern does indeed increase significantly the risk to esophageal cancer. The relative risk is even higher for those who also

consume hot tea gruel ("chagayu" in Japanese) and smoke cigarettes daily (81). The role of bracken fern toxins in the genesis of other human cancers still awaits further investigation.

Among various toxins isolated from bracken fern, shikimic acid, tannin and several flavonoids also occur at variable amounts in many different plants. For instance, among the 268 plant species examined, 158 (many of which are food plants) have been found to contain shikimic acid in various parts of the plants (15). Trace amounts of shikimic acid (up to 0.193% fresh weight) have also been detected in 56 of 83 species of Angiosperm leaf samples from Japan (82). The distribution of tannin and flavonoids in plants is discussed in Section 5.3.2.6.2 and in Section 5.3.2.6.3.

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