

# Carcinogenic effects of ptaquiloside in bracken fern and related compounds

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**Summary** Consumption of the bracken fern *Pteridium aquilinum* by cattle has been shown to induce bladder and intestinal carcinomas in cattle and to cause a number of diseases in other farm animals. An unstable glucoside named ptaquiloside, containing a reactive cyclopropane ring, has been isolated from the fern and its potent carcinogenicity proven. Nineteen of 31 ferns tested by chemotaxonomic methods in Japan have been found to contain potentially carcinogenic ptaquilosides as have *Cheilanthes sieberi* and *Pteridium esculentum*. Hydrolysis of ptaquilosides leads to pterosins; under milder conditions a dienone which is believed to be the primary carcinogen is obtained. Hypacrone, a sesquiterpene containing a reactive cyclopropane ring, has been isolated from *Hypolepis punctata* and its structure proved by synthesis. Illudins, structurally similar to ptaquiloside, have been isolated from the basidiomycete *Omphalotus illudens*. These give anti-tumour activity and similar reactivity with nucleophiles to ptaquiloside. Compound CC-1065, a highly toxic antibiotic also containing a cyclopropane ring, has been isolated from *Streptomyces zelensis*. The mechanism of its reactivity with DNA has been compared to that of ptaquiloside and the small structural differences between carcinogenic and anti-tumour activity discussed. Both CC-1065 and adozelesin, a synthetic analogue with anti-tumour activity, have been shown to alkylate the N-3 atom of adenine in a certain sequence of DNA. The reactivity of cysteine with ptaquilosides and illudins is discussed, as is the role of cysteine alkylating agents in apoptosis. © 2000 Cancer Research Campaign

**Keywords:** carcinogenic, ptaquiloside, bracken, ferns, illudins

The bracken fern *Pteridium aquilinum* is among the most common plants (Page, 1976; Taylor, 1990). Ingestion by cattle of either the whole plant or extracts leads to a syndrome in which there is panneloid bone-marrow damage, pyrexia and often gut-lining damage and ulceration (Evans et al, 1958). Also typical are widespread petechial haemorrhages (Evans et al, 1954). The presence of a carcinogen in fresh bracken ferns was first recognized over 30 years ago by Evans and Mason (1965) in trials on mice and later confirmed by other workers (Pamacku and Price, 1969; Hirono et al, 1970). Consumption of bracken fern by cattle has been shown to induce bladder and intestinal carcinomas (Fenwick, 1965; Smith, 1990) and to cause a number of diseases in other farm animals (Evans, 1979; 1986). The presence of haematuria in cattle fed bracken over long periods has been reported, together with changes of a polypous-tumorous nature in the bladder mucosa (Rosenberger, 1960). When introduced into the urinary bladders of dogs, an extract of urine from cattle fed hay in haematuria districts produced changes similar to haemangionoma. Application of the same extracts to the skin of white mice led to papilloma-type excrescences (Georgiev et al, 1963). Bracken has been identified as a farm hazard of considerable economic importance (Alonso-Amelot et al, 1995). Chemical studies of over thirty species of Pteridaceae have been carried out (Murakami and Tanaka, 1988); an unstable glucoside named ptaquiloside (Figure 1A) was isolated (Niwa et al, 1983; Van der Hoeven et al, 1983) and its potent carcinogenicity proven (Hirono et al, 1984). Ptaquiloside was shown to break down in aqueous solution in the presence of

acid, base or heat to give pterosin B (Figure 2A, R<sub>1</sub> = CH<sub>2</sub>OH, R<sub>3</sub> = H). As well as the above carcinogenic effects, other compounds present in bracken fern cause a variety of effects such as cyanogenesis and thiamine deficiency (Smith, 1997).

Three main routes may lead to human exposure to the toxic effects of bracken fern – eating the plant, physical contact with it or more particularly the spores, and ingestion of milk from affected animals. The consumption of the plant is common in Japan and, although pre-treatment with boiling water or soda ash reduces the carcinogenicity, the risk of oesophageal cancer is increased approximately by 2.1 in men and 3.7 in women (Kamon and Hirayama, 1975). Ptaquiloside has been identified in the milk produced by bracken-fed cows (Alonso-Amelot et al, 1993; 1996); the concentration in milk has been found to be about 8.5% of the amount ingested by the cow and is linearly dependent on the dose. The authors indicate that, in their view, it is ‘certainly likely’ that ptaquiloside in milk is responsible for the connection between bracken infestation and the incidence of gastric cancer in populations of farmers inhabiting cattle-range areas in Costa Rica and other countries where bracken growth is dense (Villalobos-Salazar, 1985), strongly suggesting the need to avoid the distribution of this into the food chain. Indeed neoplasia has been caused by feeding rats with the milk of cows or rats fed on a bracken fern diet (Evans et al, 1972; Villalobos-Salazar et al, 1990). A possible spatial association between bracken and cancer in England and Wales has been examined (Wells and McNally, 1989), while aerial exposure to spores may be a problem given the development of neoplasia in mice treated with spores (Evans et al, 1986; Villalobos-Salazar et al, 1995) and the observation that this treatment leads to DNA adducts in upper gastrointestinal tissues (Povey et al, 1996).

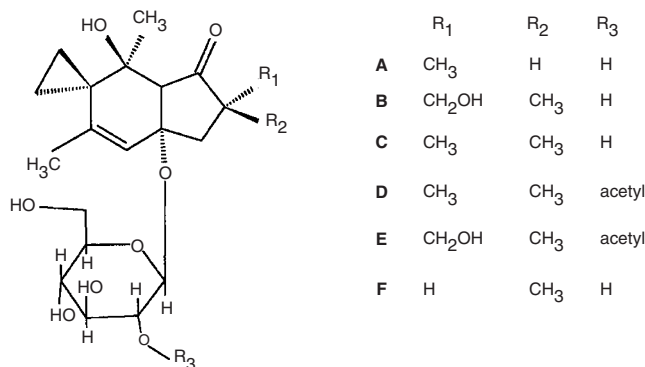
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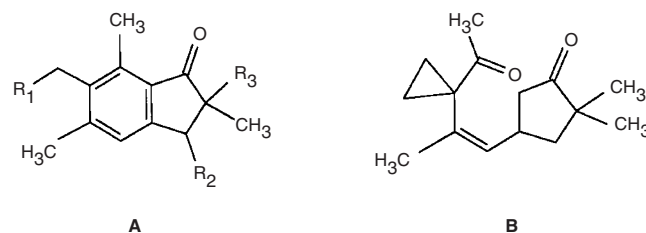
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**Figure 1** Structure of (A) ptaquiloside, (B) caudatoside, (C) methylptaquiloside, (D) hypoloside A, (E) dentoside A and (F) isoptaquiloside



**Figure 2** (A) Pterisin B and (B) hypacrone

**Table 1** Distribution of the ptaquilosides in ferns (Saito et al, 1989; Niwa et al, 1983)

Species	Ptaquiloside or ptaquiloside analogue
<i>Cheilanthes myriophylla</i>	+
<i>Cibotium harometz</i>	+
<i>Coniogramme gracilis</i>	-
<i>C. intermedia</i>	-
<i>C. japonica</i>	-
<i>Dennstaedtia distenta</i>	-
<i>D. hirsta</i>	+
<i>D. scabra</i>	+
<i>Histiopteris incisa</i>	+
<i>Hypolepis bamleriana</i>	+
<i>H. tenuifolia</i>	+
<i>H. punctata</i>	+
<i>Microlepia marginata</i> var. <i>hipinnata</i>	-
<i>M. strigosa</i>	+
<i>Monachosorum arakii</i>	-
<i>Monachosorum flagellare</i>	-
<i>Onychium japonicum</i>	+
<i>Pityrogramma calomelanos</i>	+
<i>P. sulphurea</i>	+
<i>Pteridium aquilinum</i>	+
<i>Pteris cretica</i>	+
<i>P. dispar</i>	+
<i>P. excelsa</i>	+
<i>P. fauriei</i>	-
<i>P. nipponica</i>	+
<i>P. oshimensis</i>	+
<i>P. purpureorachis</i>	-
<i>P. ryukyuensis</i>	-
<i>P. semipinnata</i>	-
<i>P. tremula</i>	+
<i>P. wallichiana</i>	+
<i>Sphenomeris chusana</i>	-

(+ corresponds to present, - to not detected)

### The distribution and structure of ptaquilosides and pterosins

The distribution of ptaquiloside in a variety of ferns has been examined using chromatography and a modified Ames test (Saito et al, 1989). Nineteen of the 31 ferns tested were found to contain the potentially carcinogenic ptaquilosides. They are listed in Table

1. Variable concentrations are present, but these can be as high as 1.3% of the dry weight (Smith et al, 1992; 1994).

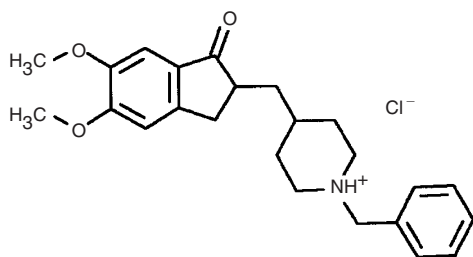
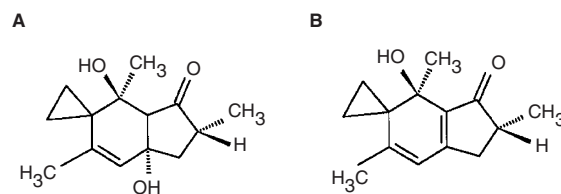
Many species of fern have been found to contain derivatives of pterisin B or related pterosins (Figure 2A). The structure of some of these are shown in Table 2 (Fukuoka et al, 1978; Ng and McMorris, 1984). *Pteridium aquilinum* has been found to contain 20 kinds of pterosins and pterosides but until recently only ptaquiloside yielding pterisin B on hydrolysis has been characterized (Saito et al, 1989). It was expected that ptaquiloside analogues would be present in *Pteridium aquilinum*, which would on hydrolysis yield pterisin Z and pterisin A. The ptaquiloside (Figure 1A), precursor to pterisin A, has recently been isolated from *Pteridium aquilinum* var. *caudatum* (Castillo et al, 1997), as has an epimer of ptaquiloside, isoptaquiloside (Figure 1F) while the precursor (Figure 1C) of pterisin Z has been isolated from *Pteridium aquilinum* subsp. *aquilinum* (Potter and Pitman, 1994). This precursor of pterisin Z has also now been isolated from *Pteridium aquilinum* var. *caudatum* (Castillo et al, 1998).

*Cheilanthes sieberi*, growing in both Australia and New Zealand, had been found to cause a syndrome indistinguishable from acute bracken poisoning; *Cheilanthes sieberi* fronds and croziers collected from both Australia and New Zealand were found to contain ptaquiloside (Smith et al, 1990; Agnew and Lauren, 1991). Analysis of the extracts for pterisin B following base and acid treatment gave concentrations twice as high as expected for the ptaquiloside alone, possibly suggesting pterisin B precursors other than ptaquiloside in *Cheilanthes sieberi* (Smith et al, 1990).

Further analogues of ptaquiloside have been isolated from other ferns including hypolosides A, B and C from *Hypolepis punctata* and B and C from *Dennstaedtia hirsta* (Saito et al, 1990), from which dennstoside A (Figure 1E) was also isolated (Koyama et al, 1991). Hypolosides B and C have the same structure as hypoloside A (Figure 1D), but with an additional p-coumaroyl group on the 4-position of the glucose. The only difference between hypolosides B and C is that they are geometrical isomers with the p-coumaroyl group existing in the *cis* and *trans* isomers respectively. Hypoloside A, B and C also yield pterisin Z on hydrolysis and dennstoside A yields pterisin A. Extraction of fresh shoots of *Hypolepis punctata* yielded hypacrone (Figure 2B) (Hayashi, 1977a; 1977b), also possessing a reactive cyclopropane ring and giving pterisin Z on hydrolysis.

**Table 2** Structures of some of the fern pterosins (Figure 2A)

Compound	Pterosin	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
a	B	CH <sub>2</sub> OH	H	H
b	O	CH <sub>2</sub> OCH <sub>3</sub>	H	H
c	F	CH <sub>2</sub> Cl	H	H
d	E	CO <sub>2</sub> H	H	H
e	Palmityl			
	Pterosin B	CH <sub>2</sub> O palmityl	H	H
f	Pterosin B	CH <sub>2</sub> O glucose	H	H
g	Pterosin C	CH <sub>2</sub> OH	OH	H
h	J	CH <sub>2</sub> Cl	OH	H
i	Acetylpteriosin			
	C	CH <sub>2</sub> OAc	OH	H
j	Pteroside C	CH <sub>2</sub> Oglu	OH	H
k	I	CH <sub>2</sub> OCH <sub>3</sub>	H	CH <sub>3</sub>
l	Z	CH <sub>2</sub> OH	H	CH <sub>3</sub>
m	H	CH <sub>2</sub> Cl	H	CH <sub>3</sub>
n	D	CH <sub>2</sub> Oglu	OH	CH <sub>3</sub>
o	Pteroside D	CH <sub>2</sub> Oglu	OH	CH <sub>3</sub>
p	A	CH <sub>2</sub> OH	H	CH <sub>2</sub> OH
q	K	CH <sub>2</sub> Cl	H	CH <sub>2</sub> OH
r	L	CH <sub>2</sub> OH	H	CH <sub>2</sub> OH
s	V	CH <sub>2</sub> OCH <sub>3</sub>	H	CH <sub>2</sub> OH

**Figure 3** Donepezil (Gopinath 1992)**Figure 4** (A) Ptaquilosin, (B) Unstable dienone

### Biological activity of pterosins and related indanone structures

Pterosins, which not only are widely occurring in ferns (Hayashi et al, 1972; Fukuoka et al, 1978; Ng and McMorris, 1984), but also in certain fungi of the class Basidiomycetes including *Fomes annosus* and *Cyathus bulleri*, have been found not to possess carcinogenic activity, although some show cytotoxicity to HeLa cells (Yoshihira et al, 1978). Several pterosins produced cytotoxic effects on the ciliate, *Paramecium caudatum* and also induced abnormal development of urchin embryos (Kobayashi and Koshimizu, 1980). Pterosin B and O have been isolated from *Pteris inequalis* and shown to be active against *Bacillus subtilis* (Kobayashi et al, 1975). Recently, a substituted indanone, structurally similar to pterosins, has been approved for the treatment of Alzheimer's disease in the USA and UK (Gopinath, 1998). This compound, developed by Eisai and co-marketed by Pfizer has the structure shown in Figure 3.

The binding-site of this compound in acetylcholinesterase (ACE) has been investigated and possible structural modifications for identifying improved ACE inhibitors for the treatment of Alzheimer's disease discussed (Pang and Kozikowski, 1994).

### Biological activity of ptaquilosides and related structures

Under weakly alkaline conditions both ptaquiloside and its aglycone ptaquilosin (Figure 4A) are converted, with D-(+) glucose liberation in the former case, into the unstable dienone (Figure 4B), which is the activated form regarded as the ultimate carcinogen (Kigoshi et al, 1993). Both the ileum and the urine of herbivores are known to be alkaline. In the field, cattle and sometimes sheep grazing on bracken fern develop tumours, most frequently in the urinary bladder. The <sup>32</sup>P-postlabelling assay has provided evidence for ptaquilosin-DNA adducts in the ileum of bracken fern-fed calves. Alkylation occurred primarily at the

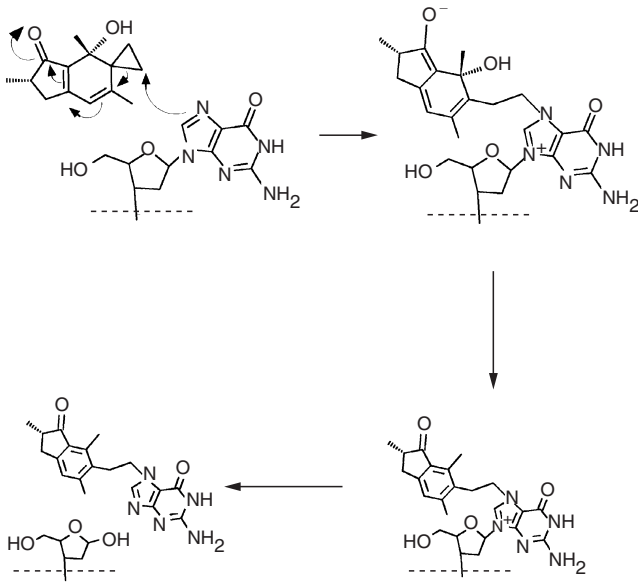


Figure 5 Part of the reaction of ptaquilosin and deoxytetranucleotide

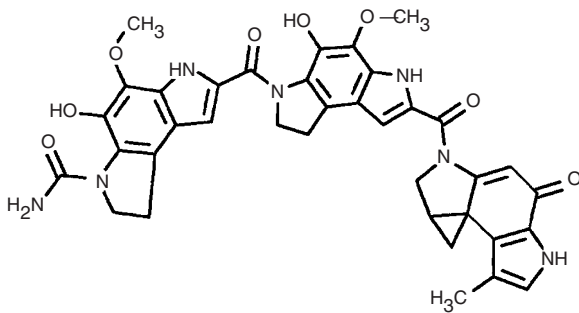


Figure 6 Compound CC-1065

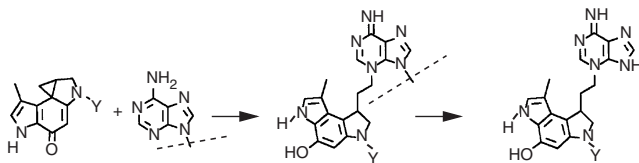


Figure 7 Reaction of CC-1065 with DNA

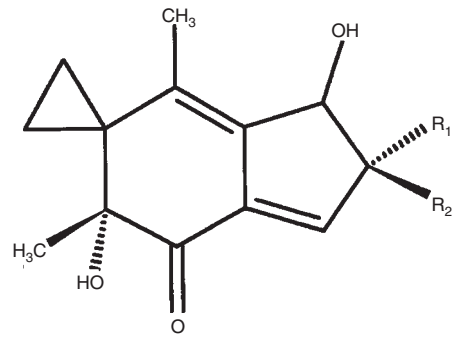


Figure 8 Illudin S ( $R_1 = \text{CH}_2\text{OH}$ ,  $R_2 = \text{CH}_3$ ) and illudin M ( $R_1 = R_2 = \text{CH}_3$ )

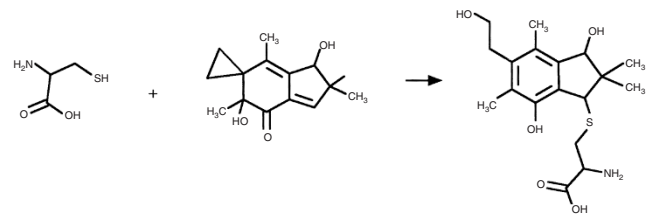


Figure 9 The reaction of cysteine with illudin M

adenine groups; the results suggest that initial alkylation of adenine by ptaquilosin (ptaquiloside) in codon 61 followed by depurination and error in DNA synthesis leads to activation of *H-ras* proto-oncogene (Prakash et al, 1996).

Ptaquilosin is strongly electrophilic and reacts readily with amino acids, nucleosides and nucleotides (Ojika et al, 1987) under mild conditions, forming covalent adducts with DNA and causing DNA strands to break (Ojika et al, 1989). In a model reaction with a deoxytetranucleotide, part of which is shown in Figure 5, the covalent adduct was found at a guanine residue and a corresponding adduct was found at an adenine residue.

Compound CC-1065, a highly toxic antibiotic containing a reactive cyclopropane ring, the structure of which is shown in Figure 6, has been isolated from *Streptomyces zelensis* (Hanka et al, 1978; Kushida et al, 1994). This compound shows significant cytotoxicity in vitro and anti-tumour activity in vivo (Kushida et al, 1994) and is significantly more toxic than actinomycin, vinblastine or maytanosine (Martin et al, 1978).

CC-1065 is believed to bind to DNA without intercalation (Swenson et al, 1981). DNA cleavage occurred through a similar mechanism to that for ptaquiloside, namely by formation of an

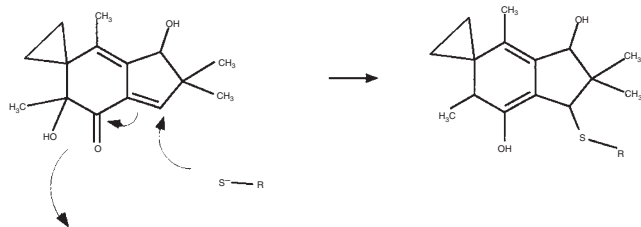


Figure 10 Reaction of illudin M with thiols

alkyladenine adduct by attack of the base on the cyclopropane in a homo-Michael addition, activated by the adjacent enone system and leading to the formation of an aromatic adduct, and then to depurination and strand breakage on heating (Figure 7) (Kushida et al, 1994). However, two significant differences have been observed. Ptaquiloside forms adducts at both guanine and adenine residues and induces the spontaneous cleavage at adenine base sites under physiological conditions, whereas CC-1065 only causes cleavage at higher temperature (70°C). At lower temperature (37°C) the adenine adduct of CC-1065 undergoes a retro-homo-Michael reaction to regenerate the initial CC-1065 structure and, it is presumed, intact DNA; the reverse reaction may require the double helical structure in DNA and be inhibited in melts (Warpehoski et al, 1992). At the same lower temperature ptaquiloside depurinates spontaneously. Adozelesin, a synthetic analogue of CC-1065 was developed with potent anti-tumour activity; both this compound and CC-1065 were found to alkylate the N-3 atom of adenine only in a certain sequence of DNA.

Illudins (Figure 8) structurally similar to ptaquiloside have been isolated from *Omphalotus illudens* and related fungi (Anchel et al, 1950; McMorris and Anchel, 1963; 1965) and have been found to be rapidly cytotoxic to haematopoietic tumours in vitro at extremely low concentrations (Kelner et al, 1987).

The illudins have been found to be quite reactive at low pH and to behave as bifunctional alkylating agents (McMorris et al, 1989). Unlike ptaquiloside, they have been found to react only with thiols including cysteine (Figure 9) at physiological pH (McMorris et al, 1990).

This reaction is thought to occur through initial attack of the thiolate at one of the enone systems in illudin to give an intermediate cyclopropane (Figure 10) which is then ring-opened by attack by solvent leading to a phenolic system (McMorris et al, 1990).

Illudin M and S failed to react with adenosine and other nitrogen nucleophiles and do not react directly with DNA. Many of these compounds are too toxic to find application, however hydroxymethylacylfulvene (McMorris, 1996) (HMAF) (Figure 11) causes complete tumour regression in all animals at the maximum tolerated dose, proving effective against breast, lung and colon cancers in animal models and in human cell cancer clones (McDonald, 1997). This compound is now available from a chemical synthesis (McMorris, 1997).

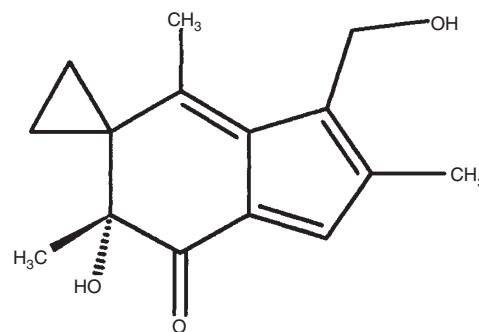


Figure 11 Hydroxymethylacylfulvene

<sup>32</sup>P-postlabelling analysis of DNA adducts formed in the upper gastrointestinal tissue of mice fed bracken extract or spores has shown that similar adducts are formed when two cyclopropane rings containing compounds 1-(4-chlorophenylsulphonyl)-1-cyclopropane and 3-cyclopropylindeno [1,2-c]pyrazol-4-(O-methyl)oxime are used instead (Povey et al, 1996). The cyclopropane rings of these compounds are also much more stable to reaction with nitrogen nucleophiles than ptaquiloside. Also it has been found that spirocyclopropane-4-piperidinones show moderate DNA cleaving activity, although again the cyclopropane rings are less strained and more stable than in ptaquilosides (Anichini et al, 1997). It is possible that these more stable cyclopropanes do not react directly with DNA. For example, the anti-cancer activity of Taxol, a compound extracted from yew trees, is mediated by tubulin polymerization and microtubule formation, thereby blocking the cell cycle in mitosis and inducing programmed cell death (apoptosis) (Wahl et al, 1996; Kinloch et al, 1999). Recently, apoptosis has become an area of intense scientific interest. This is the study of the triggers and mechanisms involved in cell death (Kinloch et al, 1999). A number of low-molecular-weight compounds have been shown to enhance or inhibit this fundamental cellular process. Decrease in the rate of apoptosis may facilitate the growth of tumours, while blocking a protein's anti-apoptotic function is of interest as potential drug targets for cancer treatment (Kinloch et al, 1999). Molecules containing a cyclopropane ring, such as ptaquilosides or the illudins, which react with thiol nucleophiles such as cysteine may act as cysteine-protease inhibitors, affecting the complex process of apoptosis giving either carcinogenic or anti-tumour activity without direct reaction with DNA. This warrants further investigation. Cysteine alkylating reagents such as N-ethylmaleimide and peptide aldehydes have been shown to be potent inhibitors of cysteine proteases inhibiting apoptosis (Nicholson et al, 1995). The control of apoptosis may lead to treatments for degenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease, immune deficiency, leukaemias and other cancers.

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