Modification and Cleavage of DNA by Ptaquiloside. A New Potent Carcinogen isolated from Bracken Fern

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The dienone (**2**), the active species chemically derived from a bracken carcinogen ptaquiloside (**1**), has been shown to bind to purine bases of salmon sperm DNA covalently and to cleave double strand supercoiled pBR 322 DNA.

Extensive studies have been carried out to obtain small molecules that can mediate DNA strand scission. Most of these molecules are designed artificially on the basis of well-known antitumour agents and possess a DNA binding moiety (DNA binder) and a reactive centre (DNA cleaver).[1,2] The search for a new type of natural product that will bind to and/or cleave DNA has become of interest recently.[3]

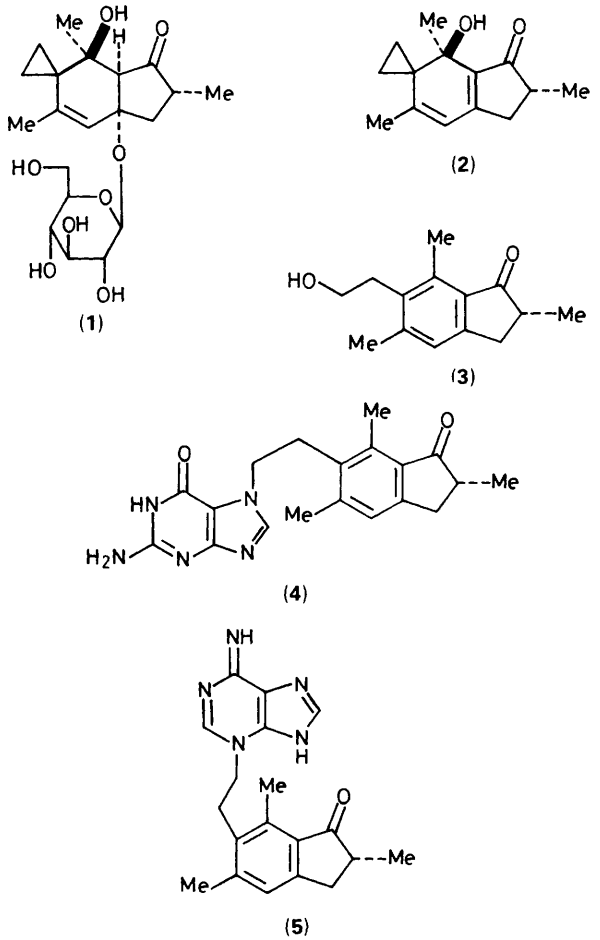
We have isolated a long-sought carcinogen ptaquiloside (**1**) from bracken fern, elucidated the novel structure,[4—6] and proved its potent carcinogenicity.[7] Under mild alkaline conditions (**1**) is converted into the unstable dienone (**2**),[4,6] which reacts with nucleophiles (*e.g.*, water) to afford stable aromatic compounds such as pterosin B (**3**),[8] which is regarded as the ultimate form of (**1**).[6] Since the covalent binding of carcinogenic alkylating agents to DNA is considered a critical event in the mechanism of action, the features in the alkylation of nucleosides and nucleotides with (**2**) have been disclosed as a preliminary experiment.[6] Here we report that this new alkylating agent (**2**) binds to purine bases of salmon sperm DNA covalently and also cleaves double strand supercoiled pBR 322 DNA under ambient conditions without metal complexation and photoirradiation.

With a view to obtaining information on the binding sites of (**2**) to DNA, isolation of the covalently binding adducts from salmon sperm DNA modified with (**2**) and their structural determination were first performed. Salmon sperm DNA (*ca.* 10 mM nucleotide concentration) was modified by incubating with (**2**) (20 mM) in aqueous acetone solution (pH 7.5, 37 °C, 2 h) and then thermally hydrolysed under neutral conditions (pH 7.0, 90 °C, 20 min).[9] During the reaction with salmon sperm DNA most of (**2**) was converted into (**3**) (*ca.* 90%), which was removed prior to the thermal hydrolysis of the (**2**)–salmon sperm DNA adducts. From the butan-1-ol soluble fraction of the hydrolysis products two modified purine bases,

Table 1. Difference UV spectra of the (**2**)–base adducts (**4**) and (**5**) and UV spectra of the corresponding methylated purine bases.[a]

	pH 1[a]		pH 7[a]		pH 13[a]	
Alkylated base	λ_{max} /nm	λ_{min} /nm	λ_{max} /nm	λ_{min} /nm	λ_{max} /nm	λ_{min} /nm
(**4**)	248 (268)[b]	235	283 (245)	261	280	257
N-7-Methylguanine	248 (267)	236	282, 246	260	278	256
(**5**)	272	236	268	245	269	246
N-3-Methyladenine	272	235	271	242	271	243

[a] The spectra of the alkylated bases were measured in H_2O–MeOH (8 : 2) containing HCl (pH 1), $MeCO_2NH_4$ (pH 7), and NaOH (pH 13), respectively. [b] UV maximum of a shoulder in parentheses.



Scheme 1

the N-7 alkylated guanine (**4**) (1.8% based on a nucleotide in DNA) and the N-3 alkylated adenine (**5**) (0.61%), were isolated by HPLC. Their structures were elucidated on the basis of the spectral (UV, NMR, and MS) data.† The site of

† *Selected spectroscopic data* for (**4**): ^{1}H NMR [270 MHz, $^2H_6$-dimethyl sulphoxide (DMSO)] δ 1.16 (3H, d, *J* 6.9 Hz), 2.30—2.65 (2H, m, AB of ABM), 2.37 (3H, s), 2.58 (3H, s), 3.14 (2H, br. t, *J* 7.4 Hz), 3.23 (1H, dd, *J* 16.7, 7.1 Hz, M of ABM), 4.25 (2H, br. t, *J* 7.4 Hz), 6.14 (2H, s, NH_2), 7.19 (1H, s), 7.80 (1H, s); MS [fast-atom bombardment (FAB), glycerol as a matrix] *m/z* 352 $(M+H)^+$. (**5**): ^{1}H NMR (270 MHz, CD_3OD) δ 1.23 (3H, d, *J* 7.3 Hz), 2.22 (3H, s), 2.41 (3H, s), 2.50—2.70 (2H, m, AB of ABM), 3.30 (1H, m, M of ABM, overlapped with solvent signals), 3.35 (2H, m, overlapped with solvent signals), 4.57 (2H, t, *J* 6.8 Hz), 7.17 (1H, s), 7.77 (1H, s), 7.99 (1H, br. s); EI MS *m/z* 335 (M^+).

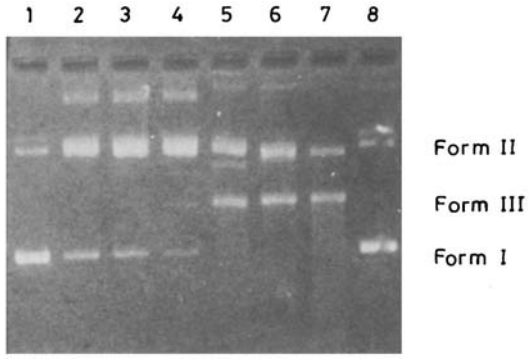


Figure 1. DNA cleavage by (**2**). Reaction mixtures containing (in a total volume of 20 μl) pBR 322 DNA (50 μM nucleotide concentration), 20 mM Tris-borate buffer (pH 7.5), 1 mM ethylenediaminetetra-acetic acid (EDTA), and 25% (v/v) acetone were incubated with (**2**) at 37 °C for 11 h. The values of [(**2**)]/[DNA nucleotide] for lanes 1—8: 0, 10, 25, 50, 100, 250, 500, 0. The samples were electrophoresed on a 1% agarose gel containing 50 mM Tris-borate buffer (pH 8.3) and 1 mM EDTA.

alkylation in guanine and adenine can be determined unambiguously, employing the difference UV spectra in acidic, neutral, and alkaline solutions by comparison with the UV spectra of the corresponding methylated guanine and adenine, respectively (Table 1).‡ The difference UV spectra were obtained by subtracting the UV spectrum of (**3**) from those of the adducts (**4**) and (**5**).

The DNA cleaving activity of (**2**) was detected by the topological changes of plasmid pBR 322 DNA as shown in Figure 1: circular supercoiled DNA (form I) was converted into nicked form II and then into linear form III. Since (**2**) reacts with DNA and also with other nucleophiles such as water and inorganic anions in the reaction mixture, high [(**2**)]/[DNA nucleotide] ratios (50—100) were required for the complete relaxation of form I DNA into form II DNA (Figure 1, lanes 4 and 5). DNA double strand scission leading to the linear form III DNA was observed, although higher [(**2**)]/[DNA nucleotide] ratios (100—500) were required (Figure 1, lanes 5—7). The very low degree of DNA cleavage with (**1**) itself (data not shown) strongly suggests that (**2**) is an active species in the carcinogenesis of (**1**).

Since the DNA cleavage with (**2**) takes place without the need for formation of metal complexes and (**2**) does not contain a phenolic moiety, which is regarded as an active site for DNA cleavage in anticancer alkylating agents such as mitomycin C[10] and CC-1065,[2,11] the cleavage mechanism with

‡ The structural determination of the nucleosides alkylated with (**2**) on the basis of the difference UV spectra was reported recently (see ref. 6).

(**2**) seems to be neither radical nor photochemical. A preliminary experiment using a 3′-^{32}P end-labelled DNA restriction fragment indicated that base-specific DNA cleavage occurred at the adenine residues: this finding makes the cleavage mechanism due to the hydrolysis of an intermediate phosphate triester improbable.

These results suggest that the alkylation at N-3 of the adenine residues by (**2**) is responsible for the DNA cleavage, while the predominant alkylation at N-7 of the guanine residues by (**2**) does not result in DNA strand scission. The role played by the indan-1-one part of the alkylated adenine residues in DNA cleavage is unknown. In this regard (**2**) is a quite novel DNA cleaver. No detailed studies other than the present work have been reported on DNA strand scission by carcinogenic natural products. Further investigation of the mechanism of DNA cleavage by (**2**) is currently in progress.

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