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A New Synthetic Analogue of the Bracken Ultimate Carcinogen: Elevation of Stability and Alteration of DNA Alkylation Site Selectivity

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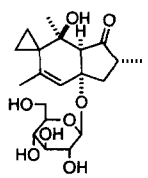
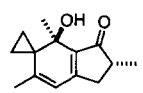
Abstract: The benzodienone **4** as a stable analogue of the bracken ultimate carcinogen (**2**) has been designed and synthesized. In the reaction with DNA the compound **4** was found to give only the N-7 alkylated product of guanine and no N-3 alkylated product of adenine and reveal guanine-selective cleavage in contrast to the natural dienone **2**. © 1997 Elsevier Science Ltd.

Ptaquiloside (**1**), a norsesquiterpene glucoside found in bracken, is a potent carcinogen[1] that covalently binds to nucleotides, causing the splitting of uncoiled DNA.[2] The dienone **2** generated from ptaquiloside (**1**) is the ultimate carcinogen and acts as a powerful alkylating agent.[3] It has been demonstrated that (1) the dienone **2** forms covalent adducts through N-3 of adenine or N-7 of guanine in DNA with opening of the cyclopropane ring in **2**, (2) spontaneous cleavage of the N-glycosidic linkage occurs primarily at the modified adenines to produce abasic sites, and (3) the abasic sites are so unstable that subsequent backbone breakage occurs via a β-elimination reaction.[2b] In the dienone **2**, the remarkably high reactivity toward adenine over guanine residues during the DNA cleavage reaction is evidently detected and the most preferable sequences for the dienone **2** is estimated to be 5'-AAAT.[2b] This preference is similar to that for CC-1065,[4] an antitumor agent. It is well documented that N-3 adenine adducts are particularly toxic unless repaired and that apurinic sites formed spontaneously from N-3 adenine adducts are rapidly repaired.[5] Accordingly, the N-7 guanine adduct of the dienone **2**, as it is with aflatoxin, may be responsible for the mutagenicity and carcinogenicity of ptaquiloside (**1**). It is of special interest to define the structural origin of the DNA alkylation selectivity caused by **1**, and hence, we explored new analogues of the native dienone **2**, the ultimate carcinogen of ptaquiloside (**1**).

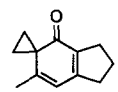
We designed the benzodienone **4** as a stable analogue of the native dienone **2**;[6] starting from the naphthalenesulfonamide **5**[7] the synthesis of the benzodienone **4**[8] was achieved (Scheme 1).[9]



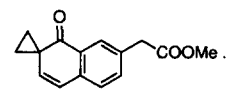
ptaquiloside (**1**)



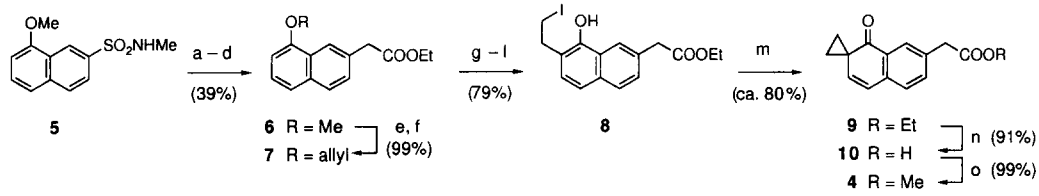
2 ($t_{1/2}$ = 11 min)



3 ($t_{1/2}$ = 3 h)



4 ($t_{1/2}$ = 46 h)



Scheme 1 (a) BuLi, THF, 0 °C, 30 min; then Li, liquid NH_3, -60 °C, 4 h; (b) MeI, BuLi, THF, 23 °C, 1 h; (c) $BrCH_2COOEt$, BuLi, THF, -78 °C, 1.5 h, 23 °C, 1 h; (d) diglyme, 150 °C, 1.5 h; (e) BBr_3, CH_2Cl_2, 0 °C, 50 min; then EtOH, 23 °C, 1 h; (f) allyl bromide, K_2CO_3, acetone, reflux, 3 h; (g) decalin, 200 °C, 1.5 h; (h) MOMCl, NaH, THF, 0 °C, 10 min, 23 °C, 2 h; (i) catalytic OsO_4, $NaIO_4$, dioxane, 23 °C, 2 h; (j) $NaBH_4$, MeOH, 23 °C, 30 min; (k) I_2, PPh_3, imidazole, toluene, 23 °C, 1 h; (l) HCl, H_2O, EtOH, 60 °C, 1 h; (m) basic Al_2O_3 (activity II-III), CH_2Cl_2, 23 °C, 10 min; (n) LiOH, H_2O, THF, 0 °C, 7 h; (o) CH_2N_2, ether, MeOH, 0 °C, 5 min.

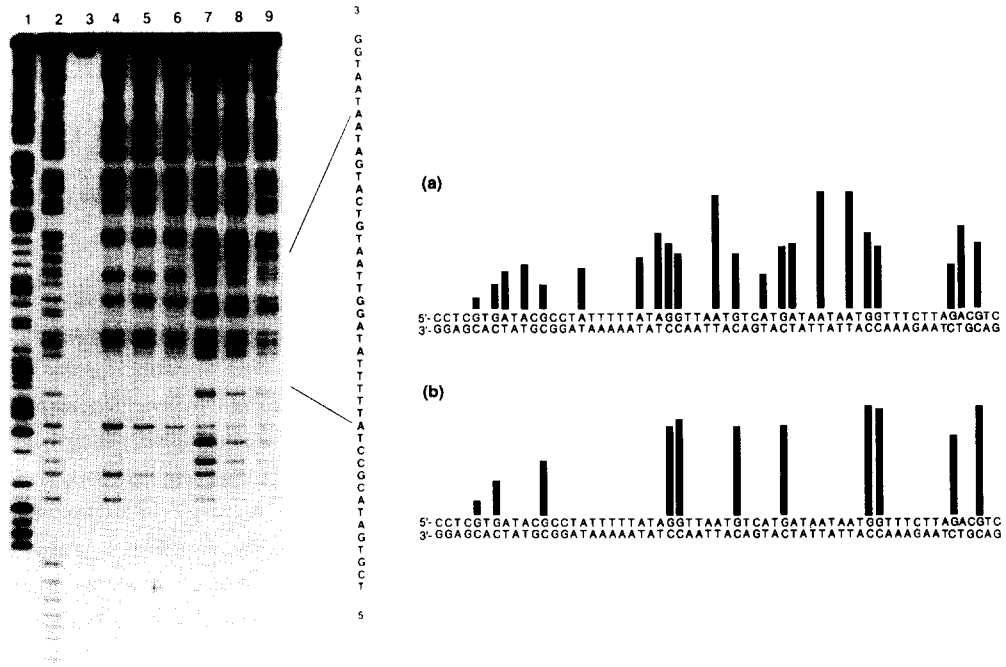
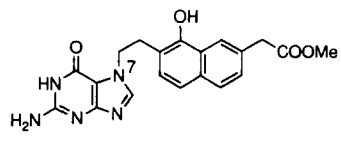


Figure 1. Nucleotide-specific cleavage by the native dienone **2** and the benzodienone **4**. The 5'-end-labeled EcoRI-SspI fragment from pBR 322 DNA was incubated with compounds **2** (lanes 7-9) and **4** (lanes 4-6). The final concentrations of the dienones were as follows: lanes 4 and 7, 500 μM; lanes 5 and 8, 250 μM; and lanes 6 and 9, 125 μM. After removal of the agent, the DNA sample was subjected to piperidine treatment. Lanes 1, 2, and 3 show the Maxam-Gilbert sequencing reactions for C+T, G+A, and intact DNA, respectively.

Figure 2. Histograms of DNA cleavage sites by the native dienone **2** (a) and the benzodienone **4** (b). The relative extent of cleavage was estimated from the densitometric scans, and the height of the bar represents relative cleavage intensity at the indicated bases.

The compound **5** was converted into the allyl ether **7** in six steps, which was subsequently transformed into the iodide **8** by a sequence of reactions involving the Claisen rearrangement reaction. The benzodienone structure was constructed by treatment of **8** with basic alumina to afford the benzodienone **9**, which led to the benzodienone **4** . The compound **4** ($t_{1/2}$ = 46 h)[10] was found to be much more stable than the native dienone **2** ($t_{1/2}$ = 11 min) and the previously synthesized artificial dienone **3** ($t_{1/2}$ = 3 h).[11] Figure 1 compares a typical autoradiographic result with the 5'-end-labeled 191-bp pBR322 DNA fragment for the DNA strand scission by the native dienone **2** and the benzodienone **4**, and the histograms of the DNA cleavage sites are given in Figure 2.[12] Apparently, the native dienone **2** induced DNA breakage at the adenine and guanine residues. In contrast, the benzodienone **4** predominantly revealed guanine-selective cleavage. The same nucleotide preference of the DNA strand scission was also obtained by using certain fragments of pBR322 DNA such as BamHI-SalI and SphI-SalI restriction fragments. The reaction of salmon sperm DNA with the benzodienone **4** was carried out in 1:3 CH_3CN-H_2O (pH 7.5) at 37 °C for 75 h. After heating at 90 °C for 15 min, the reaction mixture was subjected to HPLC analysis. Only one peak was detected and separated. This reaction product was characterized by UV, IR, 2D-NMR, and high-resolution FABMS spectra, and was determined to be the N-7 alkylated guanine **11**[13] (1.7% based on a nucleotide in DNA). No alkylated adenines were obtained in spite of the scrutiny of the reaction mixture. Previously, we reported that the reaction of DNA with the native dienone **2** followed by thermal hydrolysis afforded two modified purine bases, the N-3 alkylated adenine and the N-7 alkylated guanine.[2a] Similarly, the artificial dienone **3**[11] gave the N-3 alkylated adenine **12**[14] (1.7%) and the N-7 alkylated guanine **13**[15] (3.0%) by the reaction with DNA and subsequent thermal hydrolysis. Indeed, this compound **3** showed nucleotide-cleavage at the guanine and adenine residues quite similar to that induced by the native dienone **2**. Therefore, of special interest is the fact that the benzodienone **4** is distinctly different from both the native dienone **2** and the artificial dienone **3** in terms of nucleotide selectivity.

In conclusion, we have synthesized the benzodienone **4**, a new type of the analogue of the bracken ultimate carcinogen **2** and demonstrated that the stability is increased to a large extent and the selectivity of the DNA alkylation sites is altered in comparison with the native dienone **2**. The increasing stability of the benzodienone **4** seems to relatively enhance electrophilic reactivity toward the guanine residue in comparison with that toward the adenine residue.



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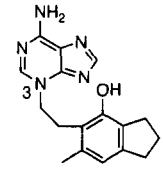

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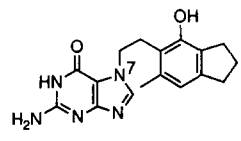

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6. The substituent (CH_2COOMe) on the benzene ring in **4** was introduced as a linker part, to which a variety of DNA binding moieties can be attached for further studies.
7. Loewenthal, H. J. E.; Gottlieb, L. *J. Org. Chem.* **1992**, *57*, 2631-2641.
8. **4**: UV (MeOH) λ_{max} 242 (ε 42000), 269 (6100), 278 (6100), 289 (3500), 351 nm (3600); IR ($CHCl_3$) 3010, 1735, 1660, 1630, 1605, 1345 cm^{-1}; ^1H NMR (270 MHz, C_6D_6) δ 8.31 (d, J = 1.6 Hz, 1 H), 7.25 (dd, J = 1.6, 7.9 Hz, 1 H), 6.96 (d, J = 7.9 Hz, 1 H), 6.38 (d, J = 9.4 Hz, 1 H), 5.27 (d, J = 9.4 Hz, 1 H), 3.26 (s, 2 H), 3.22 (s, 3 H), 1.86–1.80 (m, 2 H), 0.94–0.88 (m, 2 H); MS (FAB) *m/z* 243 $(M + H)^+$.
9. Satisfactory spectral (IR, ^1H NMR, and mass spectra) and analytical (microanalyses or high-resolution mass spectra) data were obtained for all new compounds.
10. The half-life ($t_{1/2}$) was determined in 1:3 acetonitrile-Tris borate buffer (pH 7.5) at 26 °C.
11. Kigoshi, H.; Tanaka, H.; Hirokawa, J.; Mizuta, K.; Yamada, K. *Tetrahedron Lett.* **1992**, *33*, 6647-6650.
12. The native dienone **2** or the benzodienone **4** (125–500 μM) was incubated with 0.40 μg of calf thymus DNA and a trace (<0.01 μg) of the 5'-end-labeled DNA (≈100,000 cpm) in a total volume of 20 μl. The reaction was carried out at 37 °C for 20 h in 25% acetonitrile /TBE buffer (89 mM Tris-borare, 2.5 mM Na_2EDTA, pH 8.0). When required, the sample was subsequently treated with 1 M piperidine at 90 °C for 30 min. Each lyophilized sample was dissolved in 3 μl of 90% (v/v) deionized formamide containing 1 mM EDTA and 0.01% (w/v) bromphenol blue and loaded into a 15% (w/v) polyacrylamide sequencing gel. Electrophoresis was performed at 2000 V in TBE buffer (pH 8.0). DNA sequencing was carried out using the Maxam-Gilbert method.
13. **11**: UV (1:4 MeOH–0.1 M NH_4OAc) λ_{max} 216 (ε 50000), 237 (60000), 287 (10000), 327 nm (3000); IR (KBr) 3400 (br), 2920, 1735, 1680, 1640, 1615, 1470, 1380 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1 H), 9.34 (br s, 1 H), 8.08 (br s, 1 H), 7.73 (d, J = 8.3 Hz, 1 H), 7.66 (s, 1 H), 7.33 (dd, J = 2.1, 8.3 Hz, 1 H), 7.28 (br d, J = 8.3 Hz, 1 H), 7.05 (d, J = 8.3 Hz, 1 H), 6.14 (s, 2 H), 4.37 (t, J = 8.0 Hz, 2 H), 3.83 (s, 2 H), 3.62 (s, 3 H), 3.25 (t, J = 8.0 Hz, 2 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.6, 160.2, 154.9, 152.8, 149.8, 142.9, 132.3, 130.8, 128.3, 127.4, 127.2, 125.1, 122.1, 118.8, 118.1, 107.9, 51.6, 46.1, 40.6, 31.8; MS (FAB) *m/z* 394 $(M + H)^+$.
14. **12**: UV (MeOH) λ_{max} 277 nm (ε 6200); IR (KBr) 3400 (br), 2920 cm^{-1}; ^1H NMR (270 MHz, 1:1 $CDCl_3$–CD_3OD) δ 8.02 (s, 1 H), 7.72 (s, 1 H), 6.56 (s, 1 H), 3.28 (m, 2 H), 2.83 (m, 4 H), 2.06 (quint, J = 7.2 Hz, 2 H), 1.92 (s, 3 H); MS (FAB) *m/z* 310 $(M + H)^+$.
15. **13**: UV (MeOH) λ_{max} 282 nm (ε 7400); IR (KBr) 3360 (br), 2920, 1675 cm^{-1}; ^1H NMR (270 MHz, DMSO-d_6) δ 10.77 (br s, 1 H), 8.56 (br s, 1 H), 7.64 (s, 1 H), 6.50 (s, 1 H), 6.12 (br s, 2 H), 4.24 (t, J = 7.4 Hz, 2 H), 3.02 (t, J = 7.4 Hz, 2 H), 2.75 (t, J = 7.3 Hz, 4 H), 2.07 (s, 3 H), 1.95 (quint, J = 7.3 Hz, 2 H); MS (FAB) *m/z* 326 $(M + H)^+$.

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