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DNA CLEAVAGE REACTION OF ANTITUMOUR ANTIBIOTIC, KAPURIMYCIN A3, WITH DEOXYTETRANUCLEOTIDE d(CGCG)$_2$

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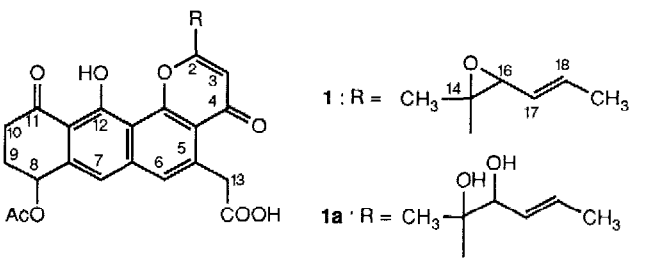
Summary: Kapurimycin A3 (kap A3, 1), an antitumour antibiotic, alkylates N7 of guanine2 (G_2) and G_4 of d($C_1G_2C_3G_4$)$_2$ to produce their covalent adducts 2 (64 %) and 3 (7.0 %), respectively. Heating at 90 °C for 5 min degraded both adducts to kap A3 - G adduct (5) with the concurrent release of their respective abasic-site containing oligomers 4 and 6.

Current interest in molecules that induce alkylation of DNA and their mechanisms of DNA damage has prompted numerous studies of antitumour antibiotics, including mitomycin C[1], anthramycin[2], CC-1065[3] and duocarmycin A.[4] Recently, kapurimycin A3 (kap A3, 1), isolated from *Streptomyces sp.* DO-115, was found to exhibit cytotoxic activity and its epoxide ring has been suggested to alkylate guanine residue of DNA.[5] We now report the detailed chemistry of kapurimycin-induced DNA cleavage by reacting this antibiotic with a self-complementary deoxytetranucleotide d(CGCG)$_2$.[6]



The reaction mixture containing **1** and d(CGCG)$_2$ in neutral pH was incubated at 0 °C Progress of reaction was monitored by reversed phase HPLC. Figure 1a shows that major (**2**) and minor (**3**) products were formed at 10.1 min and 11.2 min, respectively, after 5 h reaction. When detected at 380 nm, they remained as reduced peaks due to the antibiotic chromophore, suggesting that they may be the alkylated adducts Upon heating at 90 °C for 5 min (neutral pH), **2** was completely degraded to **4** (5.3 min) and **5** (15.2 min) (Figure 1b). Similarly, **3** produced two degraded products **6** (5.5 min) and **5**. The structure of **5** was deduced as kap A3-guanine adduct on the basis of comigration with the product isolated from reaction of calf thymus DNA and **1**.[5] Heating also caused the rapid decomposition of **1** to its 14,16-dihydroxy derivative (**1a**)[5] at 16.0 min. Products **4** and **6** were not detected at 380 nm, suggesting that they may be the modified oligomers.

Treatment of 4 with 0.2 M NaOH at 90 °C for 5 min, followed by dephosphorylation with alkaline phosphatase (A.P.) produced d(CGC). Thus 4 was a modified oligomer containing an abasic site at G_4 of $d(C_1G_2C_3G_4)_2$ (Scheme 1). Reduction of 4 with $NaBH_4$ and following treatment with snake venom phosphodiesterase (s.v. PDE) and A.P. at 37 °C for 2 h produced on HPLC dC and dG in a 1:1 ratio, together with product 7 which comigrated with the authentic sample.[7] Similar reactions of 6 identified the modified oligomer structure at G_2 of $d(C_1G_2C_3G_4)_2$. Quantitative analysis indicated that alkylation of the oligonucleotide was maximum after 40 h with the formation of 2 (64 %) and 3 (7 %) (Figure 2). Approximately 91 % of 1 had been consumed with the efficiency of oligomer alkylation being 88 %. Alkylation at G_4 of $d(C_1G_2C_3G_4)_2$ by 1 has a first-order kinetic with a rate constant of $k = 9.4 \times 10^{-6}\ s^{-1}$ at 0 °C, whereas that at G_2 was $1.0 \times 10^{-6}\ s^{-1}$. Adduct 2 was fairly stable at 0 °C with $t_{1/2}$ of 244 h.[8]

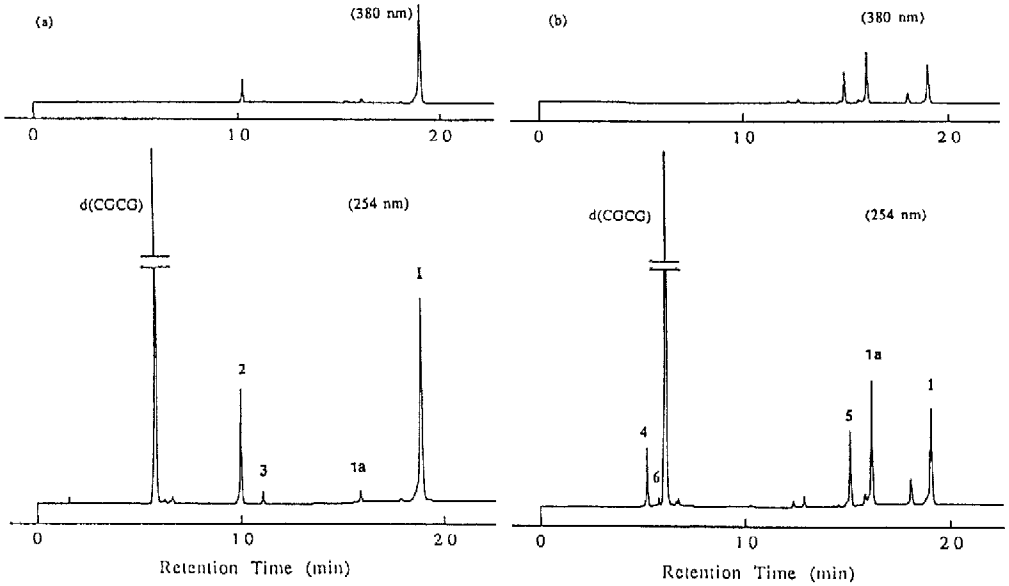


Figure 1. HPLC analysis of the reaction of 1 with $d(CGCG)_2$. The reaction mixture, incubated at 0 °C for 5 h, was analyzed (a) directly and (b) after heating at 90 °C for 5 min. Analysis was on a Cosmosil 5 C_{18} column (4.6 x 150 mm). Elution was with 0.05 M ammonium formate, 0 - 50 % acetonitrile linear gradient (20 min), at a flow rate of 1.5 mL/min. Detection was at 254 nm (lower) and 380 nm (upper) as shown.

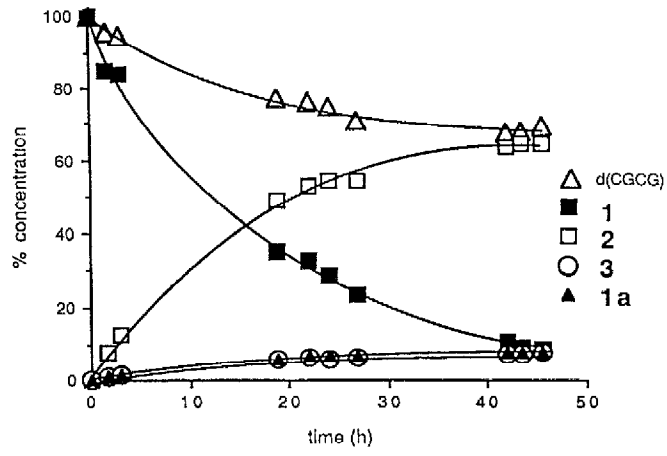
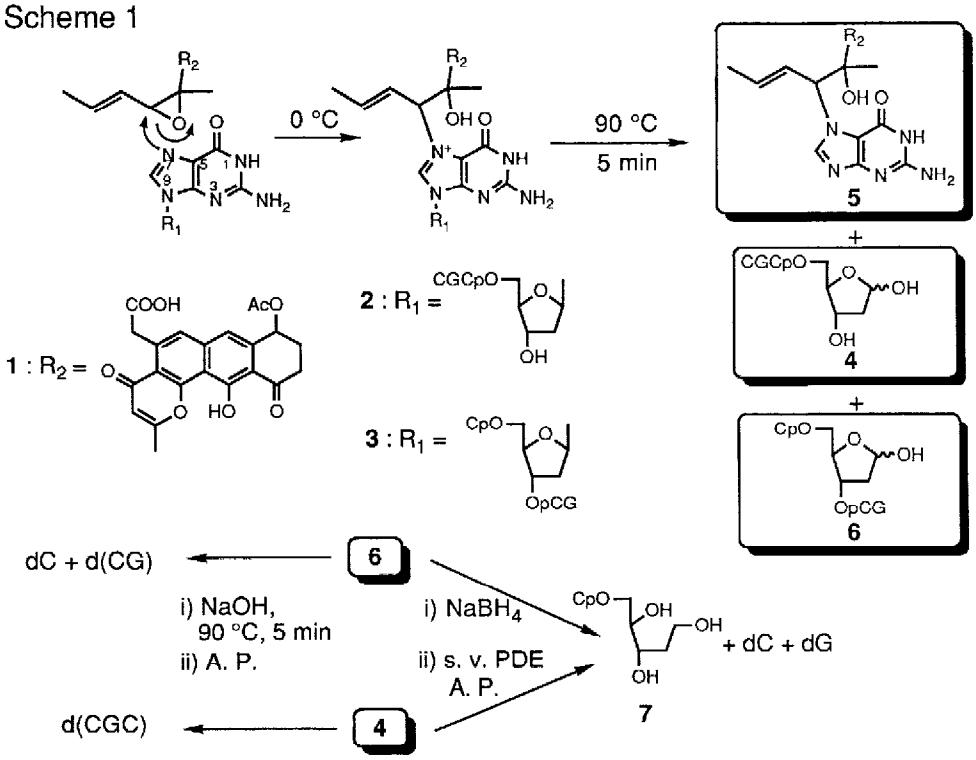


Figure 2. Time course reaction of 1 with $d(CGCG)_2$ at 0 °C. The reaction mixture (200 µL) contained 0.1 mM of 1 and 1 mM base concentration of $d(CGCG)_2$ in 50 mM of sodium cacodylate buffer (pH 7.0). Changes in % concentration were determined by HPLC peak area.

Scheme 1



Preliminary evidence for the alkylation of DNA guanine base by **1** was obtained from SIMS data of **5** and the NMR chemical shifts of H-8 and C-5 from the guanine moiety.[5] However, the evidence for the position of guanine alkylation was lacking. Therefore, methylation of **5** was conducted in order to provide evidence of the guanine alkylation site. Treatment of **5** with dimethyl sulphate in N,N-dimethylacetamide afforded two methylated adducts.[9,10] When hydrolyzed with perchloric acid, they yielded N9- and N3-methylguanines. These results provided strong evidence that the N3 of guanine in $d(CGCG)_2$ is not alkylated by **1**. Attachment at C-8 is ruled out by the presence of a one-proton singlet at 8.08 ppm in the NMR of kap A3 - G adduct.[5] Alkylation at N2 of guanine by anthramycin and mitomycin C is known to produce stable adducts without following extensive depurination and DNA damage.[11] Hitherto, no antitumour antibiotic has been reported to alkylate N1 or O6 of guanine, except for some alkylating reagents such as diazoalkanes and alkyl iodides, where such alkylations proceeded more favourably under alkaline rather than neutral conditions.[12] N1- and O6-alkylated products were usually more stable to heat and acid-catalysed hydrolysis,[12] in contrast to the marked instability exhibited by the N7-alkylated derivative.[9,12,13] In addition, N7 of guanine has been shown to be the most reactive site of nucleic acids towards alkylating agents under neutral aqueous conditions.[12,13] The isolation of N9-methylguanine and the absence of any detectable N7-alkylation product, therefore, further support that **1** was attached to N7 of guanine residue.

In conclusion, the present study provides evidence that **1** alkylates DNA at N7 of guanine to produce a thermolabile adduct which could undergo depurination to produce a more stable kap A3-guanine adduct, together with the formation of its abasic site-containing oligomer. This pathway of DNA damage may partly explain the mechanism of the antitumour action of **1**.

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5) Hara, M.; Yoshida, M.; Nakano, H. *Biochemistry* **1990**, *29*, 10449, and references therein
6) Synthesis of $d(CGCG)_2$ was conducted on an Applied Biosystem 381A DNA Synthesizer using the phosphoramidite method with 1 μmol column. Its concentration was determined by complete digestion with s.v. PDE and A.P. to corresponding 2'-deoxymononucleosides.
7) **7**; ^{1}H-NMR (D_2O, TSP) δ 1.51(ddd, 1 H, *J* = 19.9, 10.0, 4.5 Hz, 2"), 1.72 - 1.80 (m, 1 H, 2"), 2.16 - 2.24 (m, 1 H, 2'), 2.44 (ddd, 1 H, *J* = 14.1, 6.0, 3.4 Hz, 2'), 3.54 - 3.73 (m, 6 H, 5', 1", 3", 4"), 3.74 - 3.81 (m, 1 H, 5"), 3.88 (ddd, 1 H, *J* = 3.2, 6.0, 11.1 Hz, 5"), 4.06 - 4.10 (m, 1 H, 4'), 4.56 - 4.74 (m, 1H, 3'), 5.89 (d, 1 H, *J* = 7.6 Hz, 5), 6.14 (t, 1 H, *J* = 6.9 Hz, 1'), 7.67 (d, 1 H, *J* = 7.6 Hz, 6); FABMS (m/z) 410 $(M-1)^+$.
8) At 37 °C, $t_{1/2}$ of **2** was reduced to 7.5 h, whereas that at 60 °C was only 0.4 h.
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10) Methylation of **5** was performed by modification of the published method.[8] **5** (0.2 mg) was treated with 30 μL of dimethyl sulphate in 50 μL of N,N-dimethylacetamide at room temperature for 24 h. The reaction was analyzed on a Cosmosil 5 C_{18} column (4.6 x 150 mm) Elution was with 0.05 M ammonium formate, 15-35 % acetonitrile linear gradient (20 min), at a flow rate of 1.5 mL/min and detection was at 254 nm. Two methylated adducts of **5** (15.8 min), eluted at 14.6 and 16.7 min, were isolated and subsequently hydrolysed with 3 μL of perchloric acid (60 %) at 94 °C for 1 h. Each hydrolysate was next adjusted to pH 4 with 2 M NaOH before eluted isocratically with 0.05 M ammonium acetate (pH 4) at 1.0 mL/min. Hydrolysis of the methylated adduct at 14.6 min gave N9-methylguanine (24.9 min) whereas that at 16.7 min yielded N3-methylguanine (12.3 min), when compared with their respective authentic samples.
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