Pergamon

0031-9422(95)00475-0

Phytochemistry, Vol. 40, No. 5, pp. 1373–1374, 1995

0031-9422/95 $9.50 + 0.00

NMR CHARACTERIZATION OF GUANINE DNA SITE ALKYLATED BY KAPURIMYCIN A3, AN ANTITUMOUR ANTIBIOTIC FROM *STREPTOMYCES* SP.

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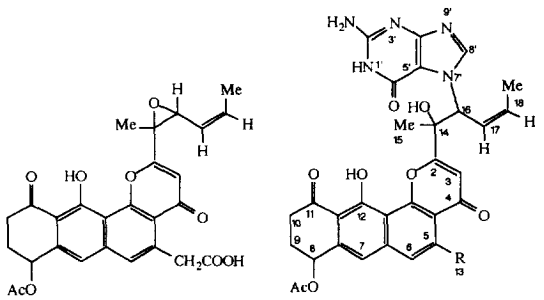
(*Received in revised form* 15 *May* 1995)

Key Word Index—*Streptomyces* sp.; kapurimycin A3; guanine DNA alkylation site; NMR.

Abstract—The kapurimycin A3–guanine adduct was formed by alkylation of the antitumour antibiotic with $d(CGCG)_2$. The site of alkylation of the guanine was confirmed by comparative NMR studies with *N*-7-methylguanine in DMSO-d_6.

INTRODUCTION

Current interest in molecules that can covalently alkylate DNA has led to numerous studies [1–4] of kapurimycin A3 (kap. A3, **1**), an antitumour antibiotic isolated from the *Streptomyces* sp. DO-115. Preliminary results from the use of synthetic self-complementary oligonucleotides have demonstrated that **1** alkylates both guanine$_2$ (G_2, 64%) and G_4(7%) of $d(CGCG)_2$ [3], and only G_4 of $d(A_1T_2C_3G_4A_5T_6)_2$, to produce their respective unstable adducts which then undergo thermal depurination to their corresponding oligomers containing an abasic site and kap. A3–guanine adducts (**2**). Evidence for the position of alkylation at the guanine by **1** has not been fully established. Hitherto, we have demonstrated indirectly by methylation and acid hydrolysis of the antibiotic–base adduct that **1** alkylates the *N*-7 position of guanine [3]. We now present NMR data to confirm the specific site of guanine alkylated by the antibiotic.



1

R
2 CH_2COOH
3 Me

RESULTS AND DISCUSSION

Previous ^{1}H NMR studies of **2** in methanol-d_4 (Table 1) showed that all of the resonances attributable to the protons from **1** were present as well as an additional singlet δ7.97 due to H-8 of guanine [2]. However, the other protons of guanine were not detected. Similarly, in the ^{13}C NMR studies of **2** in methanol-d_4, the carbon atoms of guanine were not fully assigned [2]. When **2** was dissolved in DMSO-d_6 for NMR determinations or in dimethylacetamide for methylation experiments, rapid decarboxylation of the β,γ-unsaturated δ-keto carboxylic acid moiety occurred. The HPLC-isolated decarboxylated derivative **3** showed a strong singlet at δ2.74, indicating the presence of a methyl group at C-5 and the disappearance of the H-13 doublets at δ3.84 and 4.24 present in **2**. The two singlets appearing at δ5.87 and 8.43, and not previously detected when **2** was dissolved in methanol-d_4, were assigned as OH-14 and OH-12, respectively. Furthermore, **3** showed three additional singlets at δ10.21, 6.07 and 7.99 corresponded very closely to NH-1 (δ10.68), NH_2-2 (δ6.05) and H-8 (δ7.81) of N-7-methylguanine in DMSO-d_6, respectively, thus confirming that the alkylation of guanine by **1** occurred at the N-7 position.

EXPERIMENTAL

Microorganism. The *Streptomyces* sp. was isolated from a soil sample at Kanazawa City, Ishikawa Prefecture, Japan. The culture specimen was deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, as of *Streptomyces* sp. DO-115 (accession No. FERM BP-2408).

Kap. A3 (**1**). The antibiotic was produced in a fermentation medium containing the *Streptomyces* sp., supplemented

Table 1. The ^{1}H NMR (400 MHz) data for the antibiotic–guanine adducts and *N*-7-methylguanine

H	Kap. A3–guanine adduct (**2**) (methanol-d_4) [2] δ	Decarboxykap. A3–guanine adduct (**3**) (DMSO-d_6) δ	*N*-7-Methylguanine (DMSO-d_6) δ
CH_3-14	1.68 (*s*)	1.68 (*s*)	nil
CH_3-18	1.82 (*d*) (J = 6.4 Hz)	1.82 (*d*) (J = 6.7 Hz)	nil
CH_3COO-8	2.16 (*s*)	2.17 (*s*)	nil
9	2.26 (*m*)	2.32 (*m*)	nil
9	2.37 (*m*)	2.68 (*m*)	nil
CH_3-5	nil	2.74 (*s*)	nil
10	2.95 (*m*)	2.94 (*m*)	nil
10	2.95 (*m*)	2.96 (*m*)	nil
13	3.84 (*d*) (J = 16.1 Hz)	nil	nil
13	4.24 (*d*) (J = 16.3 Hz)	nil	nil
OH-14	not detected	5.87 (*s*)	nil
18	5.92 (*m*)	5.91 (*m*)	nil
17	5.92 (*m*)	5.93 (*m*)	nil
8	6.16 (*dd*) (J = 7.5, 3.4 Hz)	6.13 (*dd*) (J = 7.4, 3.6 Hz)	nil
3	6.30 (*s*)	6.29 (*s*)	nil
16	6.43 (*s*)	6.58 (*s*)	nil
7	7.34 (*s*)	7.31 (*s*)	nil
6	7.52 (*s*)	7.56 (*s*)	nil
OH-12	not detected	8.43 (*s*)	nil
CH_3-7 (G)	nil	nil	3.81 (*s*)
NH-1 (G)	nil	10.21 (*s*)	10.68 (*br s*)
NH_2-2 (G)	nil	6.07 (*br s*)	6.05 (*s*)
8 (G)	7.97 (*s*)	7.99 (*s*)	7.81 (*s*)

with high porous polymer resin to adsorb the antibiotic, which was recovered as previously reported [1].

d(*CGCG*)$_2$. The synthesis of the self-complementary deoxytetranucleotide was conducted on an Applied Biosystem 381A DNA Synthesizer using the phosphoramidite method with a 1 μmol column. Its isolation followed the method as previously described [3].

Kap. A3-guanine adduct (**2**) *and its decarboxylated derivative* (**3**). Compound **1** was incubated with d(CGCG)$_2$ at 0° for 5 hr following the method described previously [3]. The HPLC isolated kap. A3–oligonucleotide adduct underwent depurination at 55° to form **2**. In DMSO-d_6 or dimethylacetamide, **2** was rapidly decarboxylated to **3**. HPLC isolation of **2** (15.7 min) and **3** (20.4 min) was on a Cosmosil 5 C_{18} column (4.6 × 150 mm). Elution was with 0.05 M NH_4 formate, 0–50% MeCN linear gradient (20 min), flow rate 1.5 ml min^{-1}. Detection was at 254 nm. The ^{1}H NMR signals of **2** and **3** are listed in Table 1.

REFERENCES

1. Hara, M., Mokudai, T., Kobayashi, E., Gomi, K. and Nakano, H. (1990) *J. Antibiot.* **43**, 1513.
2. Hara, M., Yoshida, M. and Nakono, H. (1990) *Biochemistry* **30**, 3597.
3. Chan, K. L., Sugiyama, H., Hara, M. and Saito, I. (1991) *Tetrahedron Letters* **32**, 7719.
4. Sugiyama, H., Chan, K. L., Hosoda, M. and Saito, I. (1991) *Nucleic Acids Symp. Ser.* **25**, 75.