Isolation and Characterization of a Novel Product, 2′-Deoxyoxanosine, from 2′-Deoxyguanosine, Oligodeoxynucleotide, and Calf Thymus DNA Treated by Nitrous Acid and Nitric Oxide

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The inactivation and mutagenic effects of HNO_2 on nucleic acids were first demonstrated around 1960.[1] The principal reaction products were identified as xanthine (Xan), hypoxanthine, and uracil.[2] Shortly thereafter, it was shown that guanine moieties (Gua) of tobacco mosaic virus RNA were not exclusively converted to Xan, but approximately half of the Gua was consumed in an unknown reaction.[3] In subsequent efforts to characterize unidentified products, 2-nitroinosine (2-nitro-Ino)[4] (maximum yield, 8.5%)[4] and cross-linked compounds[5] (maximum yield, 2.4%)[6] were isolated and identified. However, the yields of these two compounds did not fully account for the remaining half of consumed Gua. Evidently some major product(s) remain to be identified in the HNO_2−Gua systems. In the present study, we have reexamined such systems and report isolation and characterization of a new major product formed from HNO_2-treated 2′-deoxyguanosine (dGuo), oligodeoxynucleotide (dTGTT), and calf thymus DNA. Furthermore, we show that this compound is also produced in the reaction of dGuo with NO.

When 10 mM dGuo was incubated in the presence of $NaNO_2$ (100 mM) in acetate buffer (3.0 M, pH 3.7) for 2 h at 37 °C, five major peaks appeared in the reversed-phase (RP) HPLC chromatogram (Figure 1). The third peak is unreacted dGuo. 2′-Deoxyxanthosine (dXao), which is a major product of this reaction, eluted in the second peak: NMR and UV data for the isolated compound were consistent with those reported previously.[7] Xanthine produced by depurination of dXao was assigned on the basis of the agreement with the RPHPLC retention time and UV spectrum of the authentic sample. A yellowish side product eluting in the fifth peak was identified as 2-nitro-2′-deoxyinosine (2-nitro-dIno) by the EI mass fragmentation pattern, NMR spectrum, and substantial similarity of the UV spectrum to that of 2-nitro-Ino.[4]

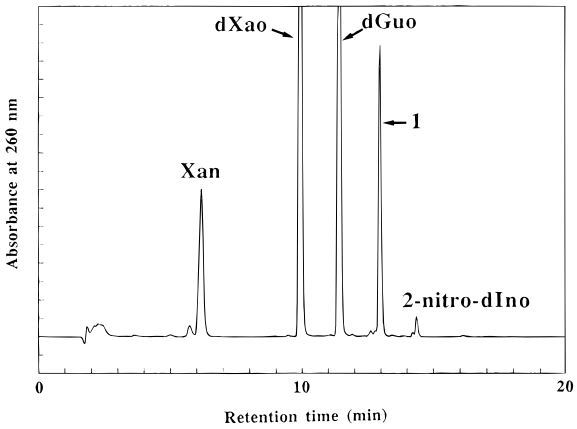

Figure 1. RPHPLC chromatogram obtained for HNO_2-treated dGuo detected at 260 nm.

The product (referred to as compound **1**) eluting in the fourth peak was isolated by preparative RPHPLC and subjected to structural assignment. Elementary analysis revealed that compound **1** has a chemical composition of $C_{10}H_{12}N_4O_5$, identical to that of dXao. High-resolution mass measurement (HR-EI) for the base fragment of compound **1** indicated that m/z = 152.032 49, which agrees with the theoretical molecular mass for the composition $C_5H_4N_4O_2$ within 1 mmu.[8] These data indicate that compound **1** and dXao are structural isomers in the base unit. In IR spectrum, however, a major band at ca. 1700 cm^{-1}, which is attributable to the stretching vibration of an amide carbonyl group and commonly observed for dXao, Xao, and dGuo, disappeared for compound **1**.[8] The ^{13}C NMR spectrum of compound **1** contained 10 resonances.[8] Five among the 10 resonances existed in the aromatic region (Figure 2). The ^1H NMR (in DMSO-d_6) showed an exchangeable singlet (7.90 ppm) attributable to two protons in addition to a set of peaks of 2′-deoxyribose and an aromatic proton (Figure 2).[8] A correlation between this singlet (7.90 ppm) and the ^{15}N signal (93.3 ppm relative to $NH_4{}^{15}NO_3$)[9] was observed in the $^1H-^{15}N$ HMQC measurement. Thus the singlet at 7.90 ppm is assigned as a primary amino group. The ^1H and ^{15}N chemical shifts of the amino group in compound **1** are fairly downfield from those of the primary amino group in dGuo (for dGuo: ^1H, 6.43 ppm; ^{15}N, 82.7 ppm). These downfield shifts suggest that compound **1** has a ring including an oxygen atom which is located near the amino group. These data imply that compound **1** is 5-amino-3-β-(2-deoxy-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,3]oxazin-7-one (2′-deoxyoxanosine). Available spectroscopic data includ-

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(8) Compound **1**. ^1H NMR (500 MHz, D_2O at 30 °C): δ (ppm/TSP-d_4) 7.98 (s, 1H, H-2), 6.26 (dd, $J_{1'2'}$ = 6.9, $J_{1'2''}$ = 6.6, 1H, H-1′), 4.61 (ddd, $J_{3'4'}$ = 4.1, 1H, H-3′), 4.12 (ddd, $J_{4'5'}$ or $J_{4'5''}$ = 3.7 or 4.9, 1H, H-4′), 3.79 (ABX, $J_{5'5''}$ = 12.4, 2H, H-5′,5″), 2.77 (ddd, $J_{2'2''}$ = 14.1, $J_{2'3'}$ = 7.1, 1H, H-2′), 2.52 (ddd, $J_{2''3'}$ = 3.9, 1H, H-2″). ^1H NMR (500 MHz, DMSO-d_6 at 30 °C): δ (ppm/TMS) 8.00 (s, 1H, H-2), 7.90 (s, 2H, NH_2), 6.05 (dd, 1H, H-1′), 5.34 (br, 1H, 3′-OH), 4.96 (br, 1H, 5′-OH), 4.34 (ddd, 1H, H-3′), 3.82 (ddd, 1H, H-4′), 3.53 (ABX, 2H, H-5′,5″), 2.49 (ddd, 1H, H-2′ or -2″), 2.23 (ddd, H1, H-2′ or -2″). ^{13}C NMR (125 MHz, DMSO-d_6 at 30 °C): δ (ppm/TMS) 159.7, 153.9, 152.6, 136.4 (C-2), 110.9, 87.7 (C-4′), 82.8 (C-1′), 70.4 (C-3′), 61.4 (C-5′), 39.8 (C-2′). The following measurements were performed for the sample further desalted by RPHPLC. IR (KBr): 3314, 3125, 2961, 2876, 2791, 2363, 1779, 1640, 1555, 1453, 1381, 1339, 1279, 1238, 1163, 1084, 1051, 1001, 951, 914, 850, 802, 764, 710, 637, 557, 444 cm^{-1}. UV: λ_{max} 248, 287 nm (pH 1), 245, 286 nm (pH 7), 266 nm (pH 13). CI (i-C_4H_{10}) m/z: 153, 117, 99, 81. HR-EI MS m/z: 152.032 49 ($M_{basefragment}$ + 1) (calcd for $C_5H_4N_4O_2$, 152.03340). Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.75; H, 4.49; N, 20.90.

(9) The chemical shift was reported downfield from the nitrogen resonance in nitrate ion of ^{15}N-enriched $NH_4{}^{15}NO_3$ in D_2O as an external standard (30.0 ppm).

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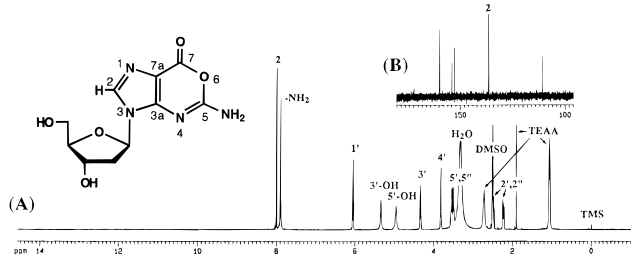

Figure 2. ^{1}H NMR spectrum (A) and aromatic region of ^{13}C NMR spectrum (B) obtained for compound **1** in DMSO-d_6 at 30 °C. The signal assignments were performed by COSY and ^{1}H−^{13}C HMQC. The numbers designated over the resonances designate the position numbers of the inset structure. Extra peaks denoted as TEAA were due to the residual triethylammonium buffer used during the purification process. Inset: Structure of 2′-deoxyoxanosine.

ing UV, IR, and NMR reported for 2′-deoxyoxanosine[10] and oxanosine,[11a] a ribonucleoside form of 2′-deoxyoxanosine, are essentially consistent with those obtained for compound **1** in this study[8] (see also supporting information). Furthermore, an equilibrium ($pK_a = 9.2$) which is attributable to saponification and relactonization of a lactone in 2′-deoxyoxanosine was observed by the pH titration of UV spectra (data not shown). From these results we have concluded that compound **1** is 2′-deoxyoxanosine.

Oxanosine was isolated as a novel antibiotic in 1981 from the culture broth of *Streptomyces capreolus* MG265-CF3 and characterized by the X-ray crystallographic study.[11] 2′-Deoxyoxanosine was synthesized from oxanosine and exhibited a stronger antineoplastic activity than oxanosine.[10] Oxanosine had weak antibacterial activity against *Escherichia coli* K-12 and *Proteus mirabilis* IFM OM-9.[11a] It also inhibited the growth of HeLa cells in culture and induced reversion toward the normal phenotype of K-*ras*-transformed rat kidney cells.[11a,12]

2′-Deoxyoxanosine was produced from dGuo (10 mM) in 21.5% yield (100 mM $NaNO_2$, 3.0 M acetate buffer (pH 3.7) at 37 °C for 6 h; the percentage yield is based on the value against the initial dGuo concentration) (Figure 3). To see if 2′-deoxyoxanosine is also generated in DNA, dTGTT (0.1 mM) and calf thymus DNA (1.0 mg/mL) were treated with HNO_2. After incubation for 6 h and subsequent enzymatic digestion with nuclease P1 and alkaline phosphatase, products were analyzed by RPHPLC. The yield of 2′-deoxyoxanosine was 24.7% for dTGTT and 29.4% for calf thymus DNA, indicating that this compound is also formed in high yield in the reaction of DNA with HNO_2.

It has been reported that dXao is also produced from dGuo by the attack of NO.[13] Therefore, possible formation of 2′-deoxyoxanosine from dGuo was explored in the presence of

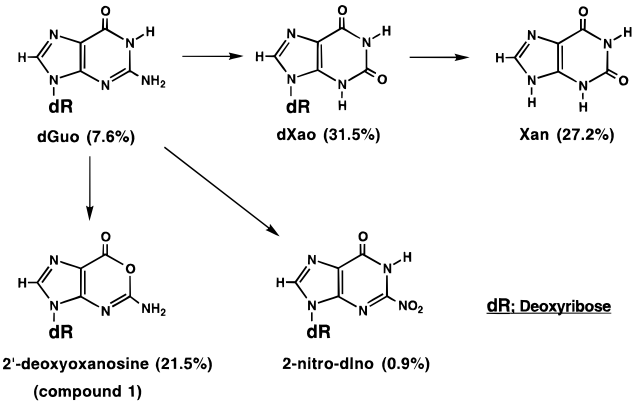

Figure 3. Reaction products of HNO_2-treated dGuo. The numbers denote the percentage yield for each compound at the reaction time of 6 h.

NO. dGuo (10 mM) was dissolved in phosphate buffer (100 mM, pH 7.0) and bubbled with NO until the pH reached 2.9. RPHPLC analysis revealed that 2′-deoxyoxanosine was formed in 11.7% yield. In another experiment, aqueous dGuo (10 mM) in 100 mM phosphate buffer (10 mL, pH 7.0) was exposed to NO gas (110 mL) in a tightly sealed vessel (inner volume of the vessel being 120 mL) at room temperature for 4 days (the final pH being 6.5). The formation of 2′-deoxyoxanosine (0.22% yield) was also observed in this system.

In conclusion, we have found a new major product in the reaction of dGuo with HNO_2 and NO by RPHPLC analysis. By spectroscopic measurements, this compound was identified as 2′-deoxyoxanosine, which was previously reported as a potent antibiotic. 2′-Deoxyoxanosine is also produced in a single-stranded oligodeoxynucleotide and double-stranded calf thymus DNA. Elucidation of genotoxic effects of 2′-deoxyoxanosine formed in DNA is an important subject of future studies.

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Supporting Information Available: HPLC conditions, quantitative procedures, characterization data of dXao and 2-nitro-dIno, comparison of spectroscopic data for 2′-deoxyoxanosine, IR spectrum, ^{1}H-^{13}C and ^{1}H−^{15}N HMQC spectra of 2′-deoxyoxanosine, and RPHPLC chromatograms of HNO_2-treated dTGTT and calf thymus DNA (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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