CHEMISTRY LETTERS, pp. 707-708, 1981.

STRUCTURE OF A NEW MODIFIED NUCLEOSIDE FORMED BY GUANOSINE-MALONALDEHYDE REACTION

Hiroshi SETO,* Kazuyuki AKIYAMA, Taisuke OKUDA,† Tsuyoshi HASHIMOTO,† Tomoyuki TAKESUE,† and Tadashi IKEMURA†

* Tokyo Metropolitan Research Laboratory of Public Health, Shinjuku-ku, Tokyo 160

† Department of Industrial Chemistry, College of Science and Technology, Nippon University, Chiyoda-ku, Tokyo 101

A new modified nucleoside was formed by the reaction of guanosine with malonaldehyde under acidic condition. This compound emitted strong yellow fluorescence and was hydrolyzed by NaOH into guanosine and malonaldehyde. Its structure was determined to be $1,N^2$-(1-propenyl-3-ylidene)guanosine by the spectroscopic analysis.

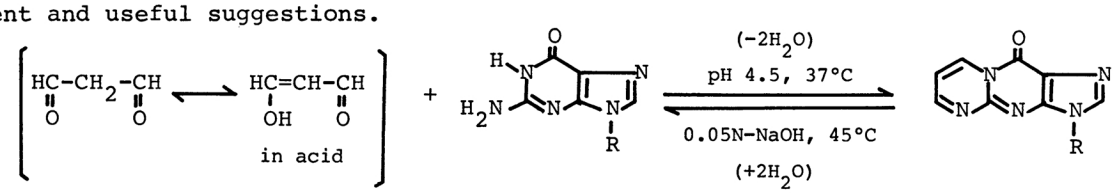
Malonaldehyde (1) has both potencies of carcinogenesis[1] and mutagenesis.[2] Although it is known that the DNA modified by (1) emits fluorescence,[3] the mechanism of interaction between DNA and (1) still remains obscure. Attempted the reaction of several nucleosides with (1), we found out a strong fluorescent compound (3), a modified guanosine. Lee et al.[4] had reported a similar fluorescent compound as simple guanosine-malonaldehyde adduct. But we obtained another result regarding the structure of the compound (3). We describe here the evidence for the proposed structure of the compound (3) which was formed by the reaction of guanosine (2) with malonaldehyde (1) under acidic condition.

The reaction of (2) with (1) in phosphate buffer solution (pH 4.5) was carried out in a stoppered flask at 37°C for 10 days. The compound (3) was isolated as yellow solid, mp>195°C (dec.), by repeated liquid chromatography. The purity of (3) was 99.7 % on HPLC analysis. The compound (3) was hygroscopic and highly soluble in water and in dimethyl sulphoxide. It (3) was partially hydrolyzed by 0.05N-NaOH into (1) and (2). The Molisch test for the detection of sugar group was positive. No carbonyl group of ketone and/or aldehyde was detected on the 2,4-dinitrophenyl hydrazine test. The FD mass spectrum of (3) indicated m/e 319 (M^+), 187 (base+H) and 133 (ribose). After the trimethylsilyl (TMS) derivative of (3) had been prepared, the EI and CI mass spectra were obtained. The molecular ion

peak of the TMS derivative was observed at m/e 535 (m/e 536, CI with methane). Therefore, the number of substitutable active hydrogens by TMS is three. The molecular formula of (3), $C_{13}H_{13}N_5O_5$, was established by high resolution mass spectrometry [M^+ 535.2099 $C_{13}H_{10}N_5O_5(TMS)_3$, calcd. 535.2102]. The IR(KBr) and UV(H_2O) spectra showed absorption bands at 3350 (O-H), 1723 (C=O) and 1630 (C=C,C=N) cm^{-1} and 215 (ε 18600), 251 (ε 13200), 308 (ε 2700), 319 (ε 3140) and 348 (ε 2750) nm, respectively. When (3) was examined in dilute solution (acetonitrile), the C=O absorption (1729 cm^{-1}) was considerably weaker than that in dry solid phase. In the fluorescent spectrum, the Ex and Em maxima were 360 and 500 nm, respectively. Its ^1H NMR spectrum (DMSO-D_6) exhibited typical AMX type signals due to the group of 1-propenyl-3-ylidene [δ 7.33 (J=3,9 and 6.8Hz), 9.08 (J=2.0 and 3.4Hz) and 9.38 (J=2.0 and 7.3Hz), each 1H, dd]. The signal of exocyclic amino group of (2) was not recognized any longer. A very broad peak [δ 5.40 (1H); this signal disappeared when D_2O was added] indicated the presence of an amide proton. There were no changes on the signals of C_8 methyne and ribose protons. Then the ^{13}C NMR spectrum showed the existence of three carbons [δ 110.5, 141.0 and 161.8 (each d)] in addition to guanosine (2) carbons. The singlet signal of C_6 was shifted to higher magnetic field in comparison with the original compound (2). In another evidence, 1-methylguanosine was unreactive to (1).

These results lead to the conclusion that the structure of the strong fluorescent compound is represented as 1,N^2-(1-propenyl-3-ylidene)guanosine (3). This compound in the solution probably form a hydrate on the N_1 atom.

We wish to thank Dr. Toshio Takido, Nippon University, for the NMR measurement and useful suggestions.



(1) (2) (3)

References

1) R.J.Shamberger,T.L.Andreone and C.E.Willis, J.Natl.Cancer Inst., 53, 1771 (1974).

2) F.H.Mukai and B.D.Goldstein, Science, 191, 869 (1976).

3) U.Reiss,A.L.Tappel and K.S.Chio, Biochem.Biophys.Res.Commun., 48, 921 (1972).

4) Y.Lee,I.Park and Y.Y.Lee, Proc.Coll.Nat.Sci.,Sect.3 (Seoul Natl. Univ.), 2, 73 (1977); C.A., 89, 142024x.

(Received March 3, 1981)