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A Novel Photoproduct of Thymine in Phosphate-buffered Saline under Far UV Irradiation

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Abstract: The photolysis of thymine in phosphate-buffered saline (PBS, pH 8.0) under the irradiation of medium pressure mercury lamp (MPML) produces a novel compound $C_5H_7N_2O_6P$. The composition and structure of the compound have been identified by elemental analysis, EI-MS, UV, IR, ^1H, ^{13}C, ^{31}P-NMR.

Keywords: UV photolysis, thymine, phosphate-buffered saline (PBS).

Exposure of DNA to UV irradiation in the range of 190-300 nm (far UV)[1] has a mutagenic effect on cellular systems. The major tow types of UV light induced damage are single-strand breakage (ssb) and base release (br). The exact mechanism leading to the damage of DNA had been investigated, although the ionization or another photoreaction of the sugar-phosphate has been proposed as a possible explanation for the higher quantum yield at 193 nm than 254 nm for single-strand breakage, most investigations both in photochemistry and radiochemistry of nucleic acids tend to deny the role of phosphate in the photolysis of nucleotides[2-3].

It has been reported[4] that the presence of phosphates altered the course of the photolysis process of pyrimidine bases and derivatives under the irradiation of low pressure mercury lamp (LPML, 254 nm), but the mechanism was not clear. In our previous papers[5-6] the photochemical actions of nucleobases and derivatives in phosphate-buffered saline undergoing MPML irradiation (continuous spectrum) were investigated and got a novel photoproduct with phosphate groups. The experimental results showed that phosphates react with nucleobases or derivatives in irradiation of MPML. In this paper we studied the photochemical reaction of 1.5×10^{-4} mol/L thymine in 0.05 mol/L phosphate solution by irradiation of MPML and found a novel compound **I (Scheme 1).**

The compound **I** has been isolated and purified by anion-exchange resin. Analytic data of the compound are as following: Elemental analysis: $C_5H_7N_2O_6P$ Calcd C 27.03%, H 3.15%, N 12.61%, P 13.96%, Found: C 27.12%, H 3.37%, N 12.41%, P 13.72%. EIMS: *m/z* $[M+H]^+$ 223(16), 179(48), 141(100), 98(32), 57(22);

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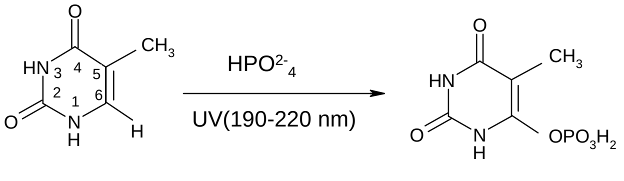
Scheme 1



I

The ultraviolet spectrum of an aqueous solution of compound **I** at pH 7.0 exhibited two maxima at 276 nm and 208 nm, with the molar extinction coefficient 3.23×10^3 and 6.34×10^4 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ respectively. ^1H-NMR (500 MHz, NaOD in D_2O): δ ppm: 1.816(s, $C\mathbf{H}_3$); ^{13}C-NMR (500 MHz, NaOD in D_2O): δ_{ppm}: 141.3(s, C-2), 132.6(s, C-4), 91.2(d, J=3.2 Hz, C-5), 132.6(d, J=6.7 Hz, C-6), 13.16($\mathbf{C}H_3$) ^{31}P-NMR (85% H_3PO_4 as external standard $\delta = 0.80$): δ_{ppm}: 0.72 (d, J=3 Hz). IR (KBr), ν cm^{-1}: 3180, 3050, 1750, 1680, 1450, 1240, 1140, 1120, 987, 950, 830.

The analysis results confirmed, that the structure of photoproduct is identical to that of compound **I**. Behrens *et al*[7] studied the ESR spectroscopy of phosphate adduct radicals obtained by reaction of photolysis H_3PO_4 with 1-methylthymine in aqueous solutions, and assigned that $HPO_4^{-}\cdot$ attacked to less hindered C(6) of 1-methylthymine and to form C(6)-phosphate adduct radicals. Our experimental results of ^{13}C-NMR of the photoproduct are coincident with the conclusions that the coupling constant (J = 6.7 Hz) of C(6) is larger than that (J = 3.2 Hz) of C(5).

Filter experiments[8] demonstrated that the wavelengths (190–220 nm) in the spectra of MPML are responsible for producing the compound **I**. Since both thymine and phosphate can absorb the UV light of wavelengths (190-220 nm), all irradiation were carried out under the condition that 80% of the light (190-220 nm) was absorbed by the phosphate dianions[8], which form to electronically excited states $(HPO_4^{2-})^*$ and released an electron to the water solvent, and then produced phosphate anion radicals $(HPO_4^{-}\cdot)$[9]. The formation mechanism of compound **I** in the photoreaction of thymine with phosphate has been not elucidated, although the result is clearly that the addition of HPO_4^{2-} is at the C(6) position of thymine. But if in acid solution (pH is lower than 5.5), the photoreactive system of thymine-PBS will not produce the compound **I**. It suggested that the reacting species are the unionized form of thymine and phosphate anion radicals $(HPO_4^{-}\cdot)$

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