A Novel Photoproduct of Uracil in Phosphate-Buffered Saline

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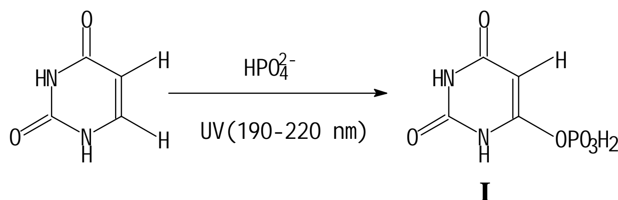
Abstract: The photolysis of uracil in phosphate-buffered saline (PBS, pH 8.0) under the irradiation of medium pressure mercury lamp (MPML) leads to the production of a novel compound $C_4H_5N_2O_6P$. The composition and structure of the compound has been identified by elemental analysis, EI-MS, UV, IR, ^1H, ^{13}C, ^{31}P-NMR.

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

Under UV irradiation the photoreaction of pyrimidine bases in aqueous solution has disclosed three types of reactions, namely: (a) nucleophilic addition of water, ethanol, or hydrogen cyanide at the 5,6-double bond; (b) dimerization by addition at the same double bond; (c) ring cleavage. It has been reported[1] 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 role of phosphate in the mechanism was not clear. In the study[2] on the damage of UV-induced DNA, researchers tend to deny the role of phosphate in the photolysis of nucleobases, nucleosides and nucleotides (NA). In previous papers[3-4] we found a new type of the photoreaction (replacement reaction) of pyrimidine bases in phosphate aqueous solutions. this paper reported the photoreaction of 1.5×10^{-4} mol/L uracil in 0.05 mol/L phosphate solution by irradiation of MPML and found the photoreactive enhancement of uracil by added phosphate, which produced a novel compound **I (Scheme 1)**

Scheme 1



The compound **I** has been isolated and purified by anion-exchange resin. The yield is 5~10%. Analytic data of the compound are as following: Elemental analysis: $C_4H_5N_2O_6P$ Calcd: C: 23.10%, H: 2.40%, N: 13.46%, P: 14.89%, O: 46.15%, Found: C: 23.41%, H:

2.31%, N: 13.72%, P: 15.01%. EIMS: *m/z* $[M]^+$ 208(18), 165(40), 127(100), 85(28), 56(33); The ultraviolet spectrum of an aqueous solution of compound **I** at pH 7.0 exhibited two maxima at 276 nm and 206 nm, with the molar extinction coefficient 8.56×10^3 and 4.36×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$ respectively. ^1H-NMR (500 MHz, $CDCl_3$): δ ppm: 7.56(d, J = 4 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ ppm: 146.3(s, C-2), 160.6(s, C-4), 101.8(d, J = 6.1 Hz, C-5), 150.9(d, J = 7.0 Hz, C-6), ^{31}P-NMR (85% H_3PO_4 as external standard $\delta = 0.80$): δ ppm: 0.85(d, J = 3 Hz). Infrared absorption spectroscopy: ν cm^{-1}**:** 3420, 3100, 1715, 1510, 1250, 1240, 1160, 1120, 995, 955.

The analysis results have indicated that the possible structure of photoproduct is identical to that of compound **I** (6-phosphoryloxy-uracil). Filter experiments[3] demonstrated that the wavelengths (190–220 nm) in the spectrum of MPML are responsible for the phosphate-induced enhancement. Since all irradiation were carried out under conditions such that 80% of the light (190-220 nm) was absorbed by the phosphate dianions, the initial process must be a transition of HPO_4^{2-} to electronically excited states $(HPO_4^{2-})^*$, then released an electron to the water solvent[5] and produced phosphate anion radicals ($HPO_4^{-}\cdot$). The formation mechanism of 6-phosphoryloxy-uracil in the photoreaction of uracil in PBS has been not elucidated, although the result is clearly the addition of HPO_4^{2-} at the C6 position of uracil. But if in acid solution (at lower than pH 5), the photoreactive system of uracil-PBS will not produce the novel photoproduct found at pH 8.0. It suggested that the reacting species are the unionized form of uracil and phosphate anion radicals ($HPO_4^{-}\cdot$).

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