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Photooxidation of acyclovir in aqueous solution

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Photodegradation of an aqueous solution of acyclovir in phosphate buffer (pH 7) under aerobic conditions was studied with light of wavelength > 270 nm. Three major products were isolated and identified on the basis of IR, NMR and mass spectral studies. The products are: (2-hydroxyethoxy)methyl spiroiminodihydantoin (**2**), (2-hydroxyethoxy)methyl(amino)-2-imino-1,2-dihydroimidazole-5-one (**3**), and 2,2-diamino-4-[(2-hydroxyethoxy)methyl]amino)-5-[2*H*]-oxazolone (**4**). Furthermore the effects of D_2O as reaction medium, addition of sodium azide and absence of oxygen on the photodegradation of acyclovir were examined, the observations indicating the involvement of singlet oxygen. The formation of the products is explained by the photooxidation of acyclovir.

1. Introduction

Phototoxicity and photosensitization by drugs is a continually growing area and has generated much research interest in the recent past. With regard to the mechanistic pathways, it is accepted that four basical paths are the main routes for phototoxic reactions, namely singlet oxygen formation, radical formation, covalent photobinding and production of photoproducts in decomposition reactions. For potential therapeutic use of drugs, knowledge about the molecular processes they undergo *in vivo* is highly significant. However the combined possibilities of these routs along with the complexities of *in vivo* systems compose a very complicated picture. Hence without ongoing help from interalia *in vitro* model research, little insight can be gained into molecular processes in photobiological effects.

Acyclovir (Ac, **1**) is structurally related to deoxyguanosine and is prone to photochemical reactions with photoactivatable chromophores and electron rich heterocyclic rings, including the electrophilic singlet oxygen. For the reasons mentioned, we investigated the photooxidation of acyclovir.

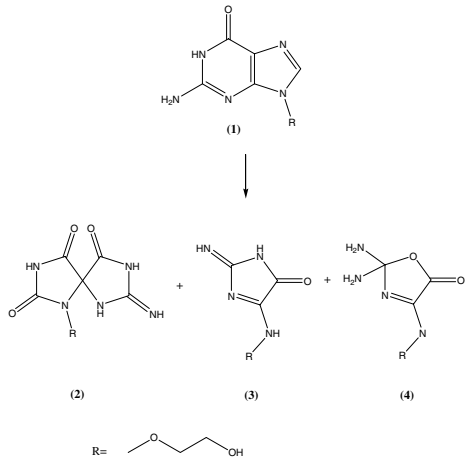
2. Investigations, results and discussion

Irradiation of an air saturated aqueous solution of Ac in pH 7 phosphate buffer using corex filtered light, followed by purification of the crude product using silica gel column chromatography, afforded three major products, which were identified by spectral studies as: **2** [(2-hydroxyethoxy)methyl spiroiminodihydantoin], **3** [4-((2-hydroxyethoxy)methyl amino)-2-imino-1,2-dihydroimidazole-5-one] and **4** [2,2-diamino-4-[(2-hydroxyethoxy)methyl) amino]-5-[2*H*]-oxazolone] (Scheme 1). The study was supplemented by irradiation in the presence of rose bengal, whereby the same products were obtained, but with considerably greater conversion in a much shorter time. When rose bengal was replaced with silica bound rose bengal (Tamagaki et al. 1980) the rate of photoxidation of Ac was slower, but it contributed to a clean workup.

A brown colouration was observed for compound **4** after spraying the silica gel TLC plates with hydroxylamine iron(III) chloride, suggesting the presence of a lactone moiety in compound **4**. The photomediated transformation of Ac involves singlet oxygen, evidenced by the following observations: (1) no loss of substrate was observed when oxygen was excluded from the medium; (2) destruction of substrate was completely suppressed in the presence of sodium azide, a singlet oxygen quencher; (3) loss of substrate was accelerated in D_2O, which is well known to be a singlet oxygen life time promoter; (4) the enhanced degradation in D_2O was also inhibited by sodium azide.

A comparison of ^{1}H NMR and ^{13}C NMR spectra of Ac and those of the photoproducts **2**, **3** and **4** did not show any significant change in the chemical shifts of protons

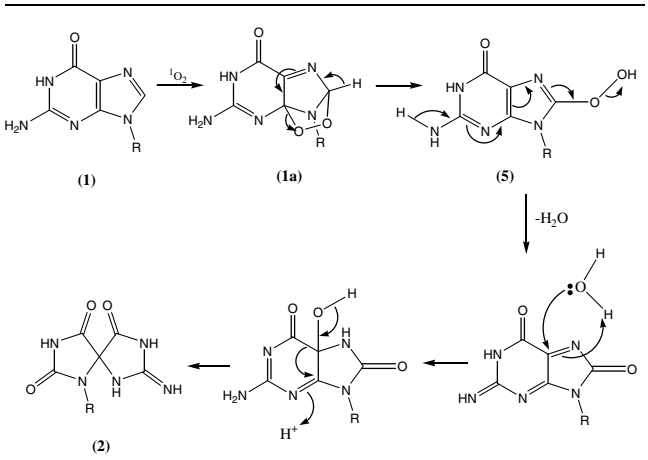
Scheme 1



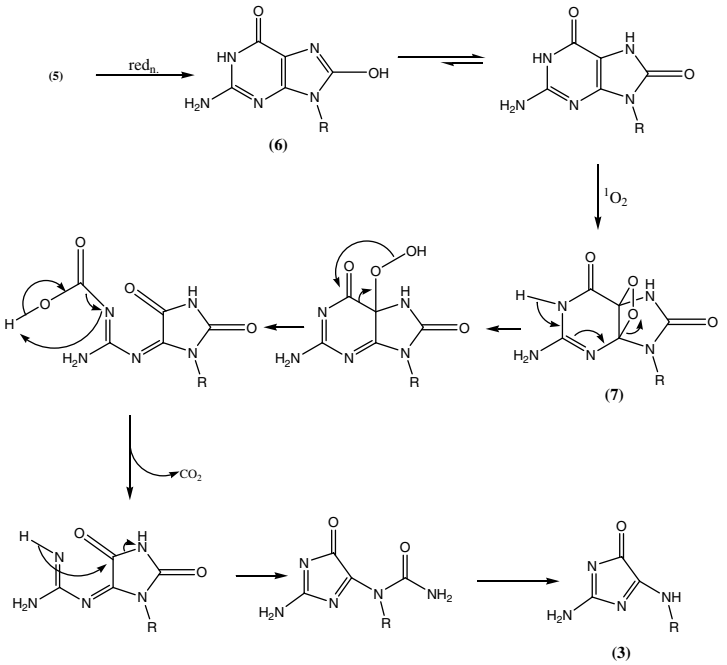
and carbon atoms of the side chain. The slight up field shift observed for methylene protons of the side chain with respect to Ac may be explained by loss of aromaticity of the heterocyclic ring. The lack of an 8-H resonance signal (δ 8.05) in the low field region of the ^{1}H NMR spectrum indicates that the purine ring of the substrate has been modified. In the ^{13}C NMR spectrum of compound **2**, a signal at δ 181.9 was assigned as an imine type carbon C-8 and signals at δ 165.8, 172.5 and 171.3 ppm were attributed to carbonyl carbons at C-2, C-4 and C-6 respectively. One important feature of the ^{13}C NMR spectrum is the appearance of a new resonance signal at 85.1 ppm, which was assigned to the quaternary carbon in the spiro ring in comparison with the spectra of related spirohydantoins (Modric et al. 1994; Johnson et al. 1994). The presence of a hydantoin ring in product **2** is evidenced by its IR spectrum, which showed a sharp absorption band at 1800 cm^{-1} along with three intense absorption bands in the 1780–1700 cm^{-1} (Poje et al. 1980). Inspection of the low field region of the ^{13}C NMR spectra of the two photoproducts **3** and **4** revealed the loss of two carbon atoms in the starting compound. Resonance signals at δ 8.20 for **4** and δ 9.31 for **3**, exchangeable with D_2O, were assigned to those of 4-NH for **4** and 5-NH for **3**, respectively. This observation is consistent with the opening of the imidazole ring of Ac. In the case of product **3**, two other exchangeable protons observed at δ 9.08 and 8.90 were assigned to 2-NH and 3-NH respectively. In the spectrum of **4**, a broad signal at δ 7.58 is equivalent to four exchangeable protons of two amino groups on sp^3 carbon C-2.

The structure of the three products identified in this study is also well supported by their mass spectra. A base peak at m/z 258 corresponds to the $[C_8H_{11}N_5O_5 + H)]^+$ ion of oxidation product **2** and a peak at m/z 184 corresponds to protonated spiroiminodihydantoin, the expected consequence of fragmentation involving the loss of a (hydroxyethoxy) methyl unit. The spectrum of compound **3** exhibits a major peak corresponding to the protonated molecule $[C_6H_{10}N_4O_3 + H]^+$ at m/z 187 and an other peak at m/z 113 $[C_3H_4N_4O + H]^+$ arising from loss of a (hydroxyethoxy) methyl unit followed by protonation. The mass spectrum of compound **4** recorded the presence of the molecular ion $[C_3H_{12}N_4O_4 + H]^+$ at m/z 205 and the fragmentation ion $[C_3H_6N_4O_2 + H]^+$ arising from splitting of the side chain at m/z 131. The two other fragments, $[C_6H_{12}N_4O_4{-}CO_2 + H]^+$ at m/z 161 and $[C_3H_6N_4O_4{-}CO_2 + H]^+$ at m/z 87, may be rationalized by the release of CO_2 from the molecular ion and the $[C_3H_6N_4O_2 + H]^+$ fragment respectively. A similar mass fragmentation pattern has already been described for imidazolone-2′-deoxyribonucleoside and oxazolone-2′-deoxyribonucleoside (Martinez et al. 2002; Ravanat et al. 2000). The three products identified in this study as spiroiminodihydantoin (**2**), imidazolone (**3**) and oxazolone (**4**) are analogous in structure to the products described for photooxidation of deoxyguanosine and deoxyguanosine derivatives. (Cadet et al. 1997; Ravanat et al. 1995; Ravanat et al. 2000; Adam et al. 2002; Martinez et al. 2002).

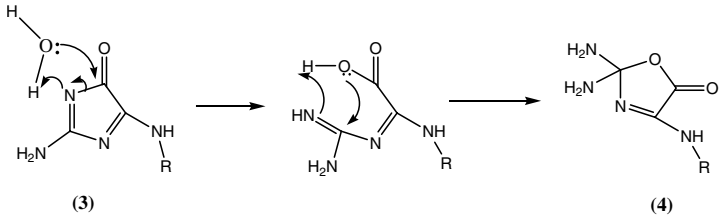
Scheme 2



Scheme 3



Scheme 4



The formation of photoproducts **2**, **3** and **4** has been rationalized as depicted in Scheme 2. The reaction of dienophile $^1O_2$ with the guanine moiety of Ac by a Diels-Alder [4 + 2] reaction involving the C-4 and C-8 carbons of the purine ring results in the formation of 4,8-purine endoperoxide (1a), which isomerizes to the 8-hydroperoxyderivative (5). This resulting hydroperoxide may undergo dehydration followed by hydrolytic cleavage to form spiroiminodihydantoin **2** (Scheme 2). Additionally, the hydroperoxide **5** in its reduced form **6** undergoes further [2 + 2] cycloaddition to produce an unstable dioxetane, which on subsequent decomposition gives the imidazole product **3** (Scheme 3). The sequential formation of imidazolone from unstable dioxetane has a precedent in the similar singlet oxygen photooxidation of nucleosides (Buchko et al. 1995; Raoul and Cadet 1996). The initially generated photoproduct, namely imidazolone **(3)**, leads on hydrolysis to the formation of the oxazolone derivative **4** (Scheme 4).

3. Experimental

3.1. Apparatus

Irradiations were carried out in a photoreactor equipped with a medium pressure mercury vapour lamp (Philips, 450 W) inserted in a water-cooled immersion well with a continuous supply of water. The incident photon flux of the irradiation setup was 9.78×10^{-7}einstein/min, as determined using ferrioxalate actinometry (Hatchard and Parker 1956). IR spectra were recorded with KBr discs on a Perkin Elmer model RX1 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-300 spectrometer using $SiMe_4$ as internal standard. FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using *m*-nitrobenzyl alcohol (NBA) matrix and argon as FAB gas. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage.

3.2. Chemicals

All chemicals used were of analytical and pharmaceutical grade. Acyclovir was extracted from the commercial medicinal product Acivir (Cipla Limited, Mumbai, India) with a soxhlet extractor using methanol as the solvent, purified by TLC and recrystallized from the same solvent. Melting point, ^{1}H NMR and co-TLC were compared with the authentic pure sample to determine the purity of the acyclovir.

3.3. Photooxidation procedure

Irradiation of an aqueous solution of Ac (5 mM) in 50 mM phosphate buffer (pH 7) was carried out both in the absence and the presence of rose bengal (0.1 mM) as sensitizer with a medium pressure mercury vapour lamp. The solution was continuously stirred during photolysis and the temperature of the solution was kept at 15 °C during irradiation by cooling with a water streamer immersed in the solution. A corex filter transmitting above 270 nm was used for photolysis of Ac. Progress of the reaction was monitored by TLC. After 6 h of irradiation, removal of solvent in a rotary evaporator (30 °C) and silica gel column chromatography (chloroform : methanol) of the photolysate yielded compounds **2**, **3** and **4** as products. The above photoreaction was also examined in deuterium oxide and in the additional presence of sodium azide (1.0 mM), to estabilish the involvement of singlet oxygen in this photoreaction.

3.4. Characterization of products

Compound **2**: yield 85.50 mg (38%); HRMS calcd. For (M^+) $C_8N_5O_5H_{11}$ 257.2054, found 257.2057; IR(KBr): 3490, 3475, 3365, 3320, 1800, 1760, 1735, 1700, 1150 cm^{-1}; ^{1}H NMR (DMSO, δ, ppm): 8.43, 8.38, 8.35, 8.27 (4 H, NH), 5.24 (d, 2 H), 3.71 (t, 2 H), 3.56 (t, 2 H); ^{13}C NMR (DMSO, δ, ppm): 181.9 (C-8), 172.5 (C-4), 171.3 (C-6), 165.8 (C-2), 85.1 (C-5), 69.7, 67.9, 61.1; FAB-MS m/z: 258 $[C_8H_{11}N_5O_5 + H]^+$, 184 $[C_5H_5N_5O_3 + H]^+$.

Compound **3**: yield 20.25 mg (9%); HRMS calcd. For (M^+) $C_6H_{10}N_4O_3$ 186.1700, found 186.1698; IR(KBr): 3240, 1734, 1645, 1528, 1155 cm^{-1}; ^{1}H NMR (DMSO, δ, ppm): 9.08 (2-NH), 8.90 (3-NH), 9.31 (5-NH), 4.73 (d, 2 H), 3.70 (t, 2 H), 3.56 (t, 2 H); ^{13}C NMR (DMSO, δ, ppm): 184.8 (C-2), 176.7 (C-4), 166.5 (C-5), 69.8, 69.2, 61.1; FAB-MS m/z: 187 $[C_6H_{10}N_4O_3 + H]^+$, 113 $[C_3H_4N_4O + H]^+$.

Compound **4**: yield 24.75 mg (11%); HRMS calcd. For (M^+) $C_6H_{12}N_4O_4$ 204.1840, found, 204.1845; IR(KBr): 3350, 2945, 1780, 1735, 1659, 1490, 1160 cm^{-1}; ^{1}H NMR (DMSO, δ, ppm): 7.58 (NH_2, 4 H), 8.20 (4-NH), 4.73 (d, 2 H), 3.70 (t, 2 H), 3.56 (t, 2 H); ^{13}C NMR (DMSO, δ, ppm): 166.3 (C-2), 156.1 (C-4), 160.1 (C-5), 69.9, 69.8, 61.1; FAB-MS m/z: 205 $[C_6H_{12}N_4O_4 + H]^+$, 131 $[C_3H_6N_4O_2 + H]^+$, 161 $[C_6H_{12}N_4O_4 - CO_2 + H]^+$, 87 $[C_3H_6N_4O_2 - CO_2 + H]^+$.

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