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# Radiation-induced reduction of quinoxalin-2-one derivatives in aqueous solutions

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## HIGHLIGHTS

- Rate constants $k(e^-_{aq} + Q/3-MeQ)$ have been determined at 25 °C at two pHs (7 and 13).
- Equilibrium constants ($pK_a \geq 13.5$) for the protropic equilibria have been provided.
- Spectral characteristics for $Q^{\bullet -}$/3-$MeQ^{\bullet -}$ and $QH^{\bullet}$/3-$MeQH^{\bullet}$ have been determined.
- The most stable hydrogenated radicals ($QH^{\bullet}$ and 3-$MeQH^{\bullet}$) have been defined.

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## ABSTRACT

Quinoxaline-2-one derivatives have been proposed as potential drugs in treatments of various diseases since some of them showed a variety of pharmacological properties. The kinetics and spectral characteristics of the transients formed in the reactions of hydrated electrons ($e^-_{aq}$) with quinoxalin-2-(*1H*)-one (Q) and its methyl derivative, 3-methyl quinoxalin-2-(*1H*)-one (3-MeQ) were studied by pulse radiolysis in aqueous solutions at pH ranging from 5 to 14. The transient absorption spectra recorded in the reactions of ($e^-_{aq}$) with Q and 3-MeQ at pH 7 consisted of a broad, almost flat band in the range 390–450 nm and were assigned to the respective protonated radical anions ($QH^{\bullet}$/3-$MeQH^{\bullet}$) at N4 atom in a pyrazin-2-one ring. On the other hand, the transient absorption spectra recorded in the reactions of ($e^-_{aq}$) with Q and 3-MeQ at pH 13 are characterized by a broad band with a much better pronounced maximum at $\lambda_{max}=390$ nm and higher intensity (in comparison to that at pH 7) and were assigned to the respective radical anions ($Q^{\bullet -}$/3-$MeQ^{\bullet -}$). Both forms are involved in the prototropic equilibrium with the $pK_a$ located at pH ≥ 13.5. The rate constants of the reactions of ($e^-_{aq}$) with Q and 3-MeQ were found to be at pH 7 $(2.6 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$ and $(2.1 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$ and at pH 13 $(1.6 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$ and $(1.3 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$, respectively. Semi-empirical quantum mechanical calculations reproduce fairly well the spectral features of the experimental absorption spectra and show that protonated radical anions at nitrogen atom (N4) in both molecules are the most stable hydrogenated radicals.



## 1. Introduction

Quinoxaline-2-one derivatives are particularly interesting since some of them showed a variety of pharmacological properties, such as antimicrobial (Ajani et al., 2010; El-Sabbagh et al., 2009; Sanna et al., 1998, 1999), antiviral (Xu et al., 2009), antifungal (Carta et al., 2002; Ingale et al., 2007; Sanna et al., 1999), anxiolytic (Ulrich et al., 1998), analgesic (Ingale et al., 2007), antiinflammatory (El-Sabbagh et al., 2009), antithrombotic (Ries et al., 2003; Willardsen et al., 2004), and antitumor (Hirai et al., 2011; Koth et al., 2007; Lawrence et al., 2001; Meyer et al., 2006) activities. Based on the computer modeling and "in vitro" studies, quinoxalin-2-ones have been proposed as potential drugs in treatments of various diseases (Carta et al., 2006). The Structure Activity Relationship (SAR) studies have revealed that quinoxalin-2-ones derivatives bound to proteins receptors (e.g. in Cdk) are generally located close to the adenosine triphosphate (ATP) binding pocket (Hirai et al., 2011; Mori et al., 2008). The fact that these compounds are bound in very specific position in proteins may have serious consequences in their interactions with either amino acid residues or radicals derived from them. Certain amino acids residues – tyrosine (Tyr), tryptophan (Trp),

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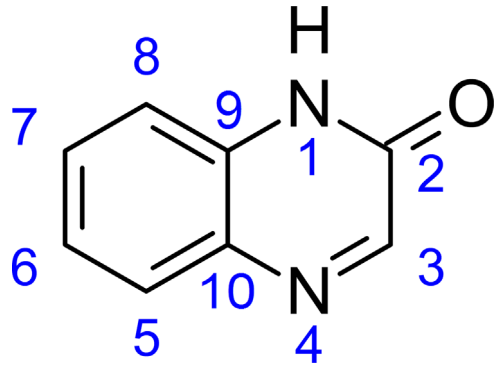


**Chart 1.** General structure of quinoxalin-2-ones.

and cysteine (Cys) are particularly vulnerable to oxidation. Therefore, the radical cations derived from quinoxalin-2-ones can modify these amino acids that are reasonably good electron donors and can be oxidized to tyrosyl (TyrO$^{\bullet}$), tryptophyl (TrpN$^{\bullet}$), and thiyl (CysS$^{\bullet}$) radicals, respectively. On the other hand, these radicals are reasonably good electron acceptors and can potentially act as oxidants of quinoxalin-2-ones intercalated in a protein matrix.

A key factor that is decisive in their biological activity is substitution at the carbon-3 in the pyrazine ring and at the carbons 6 and 7 in the benzene ring of the primary quinoxalin-2-one structure (Chart 1). Nearly all biologically active derivatives are substituted in those specific positions.

In order to address mentioned above oxidation processes involving quinoxalin-2-one derivatives, the kinetics and spectral characteristics of the transients formed in the reactions of $^{\bullet}$OH and $^{\bullet}N_3$ radicals with quinoxalin-2(1H)-one (Q) and 3-methyl-quinoxalin-2(1H)-one (3-MeQ) were studied by pulse radiolysis in aqueous solutions at pH 7. A primary distribution of the $^{\bullet}$OH attack was found nearly equal between benzene and pyrazin-2-one rings. Moreover, oxidation of Q and 3-MeQ by $^{\bullet}N_3$ with the rate constants similar to those measured for $^{\bullet}$OH suggests their rather low oxidation potential (Skotnicki et al., 2014).

Surprisingly, there are only a few reports about radical reduction processes involving quinoxalin-2-one derivatives. Some derivatives were found to initiate free radical polymerization by electron transfer from N-phenylglycine (Kucybala and Paczkowski, 1999; Kucybala et al., 2000). Their photoreduction by amines leads to the corresponding stable products dihydroquinoxalin-2-ones or the reductive dimers depending on the substituent in position 3 of the pyrazine ring (Nishio, 1990).

The photophysical and photochemical behavior of 1-methyl-3-phenyl-quinoxalin-2-one and 3-phenyl-quinoxalin-2-one in the presence of amines has been reported in some selected organic solvents (acetonitrile, methanol and hexane) (De la Fuente et al., 2002, 2000). Spectral and kinetic characteristics of the intermediate species: triplet ion-radical pairs ($Q^{\bullet -}$/amine$^{\bullet +}$) and hydrogenated neutral radicals (QH$^{\bullet}$) have been obtained by laser flash photolysis (De la Fuente et al., 2002). Calculated spectra using quantum mechanical semi-empirical AM1, PM3, and ZINDO/S approach were found to be in agreement with the experimental spectra (De la Fuente et al., 2002). Recently, the stable products resulting from the photoreduction of six 7-substituted-3-methyl-quinoxalin-2(*1H*)-one derivatives (substituents: $H_3CO$–, $H_3C$–, F–, H–, $CF_3$–, CN–) by α-amino-type radicals derived from N-phenylglycine (PhNHC$H_2^{\bullet}$) in acetonitrile solutions have been identified. Moreover, spectral and kinetic characteristics of the intermediate species: triplets ($^3$Q), radical anions ($Q^{\bullet -}$) and hydrogenated neutral radicals (QH$^{\bullet}$) have been obtained by laser flash photolysis in the presence of DABCO and N-phenylglycine (NPG) (De la Fuente et al., 2013).

The electrochemical studies of three 3-methyl-quinoxalin-2(1H)-ones: 3-methyl-quinoxalin-2(1H)-one, 3,6,7-trimethylquinoxalin-2(1H)-one and 7-amino-3-methyl-quinoxalin-2(1H)-one showed that the pyrazine ring is the electroactive center undergoing two-electron reduction (Zimpl et al., 2012).

Examples given above strongly indicate a necessity to gain a comprehensive and systematic knowledge about spectral and kinetic properties of radicals and radical ions derived from quinoxalin-2-ones. In particular, there is no information about the radicals and radical anions forming during reduction of these compounds. Therefore, in the current work we have investigated reduction processes in quinoxalin-2(1H)-one (Q) and its methyl derivative, 3-methyl-quinoxalin-2(1H)-one (3-MeQ) in aqueous solutions at pH ranging from pH 4 to 14, and starting from the primary transients formed by radiation-induced reduction by hydrated electrons ($e^-_{aq}$). These studies comprise spectral and kinetic properties of transients formed, including an assessment of the $e^-_{aq}$ reactivity by determination of the respective second-order rate constants with quinoxalin-2-ones.

## 2. Materials and methods

### 2.1. Materials

Quinoxalin-2(1H)-one (2-quinoxalinol) (Q) was purchased from Aldrich and used without further purification. Nitrous oxide ($N_2O$) >98% was from Messer, Poland. 3-Methyl-quinoxalin-2(1H)-one (3-methyl-2-quinoxalinol) (3-MeQ) was prepared by the classical reaction of the corresponding o-phenyldiamine (1 mmol) by adding drop by drop methyl pyruvate (1.2 mmol) and triethylamine (3 mmol) in ethanol. A detailed description of the synthesis, purification and spectral characterization has been given elsewhere (De la Fuente et al., 2013).

All solutions were made with triply distilled water provided by a Millipore Direct-Q 3-UV system. The pH was adjusted by the addition of either $HClO_4$ or NaOH. Prior to irradiation, *tert*-butanol (0.5 M) was added to the samples which were subsequently purged gently with Ar for 30 min per 50 mL volume before experiments. The typical concentration of solutions in pulse radiolysis experiments was 0.1 mM of quinoxalin-2-ones unless otherwise specified.

### 2.2. UV–vis spectrophotometry

The absorption spectra were recorded with the JASCO V-670 UV–vis spectrophotometer using a 1 cm optical path length cell. Water without additives was used as a reference sample. An aliquot of 1 ml was taken to measure the absorption spectrum.

### 2.3. Pulse radiolysis

Pulse radiolysis experiments were performed with the INCT LAE 10 MeV linear accelerator with a typical pulse length of 8 ns. A detailed description of the experimental setup has been given elsewhere along with the basic details of the equipment and the data collection system (Bobrowski, 2005). Absorbed doses per pulse were on the order of 20 Gy (1 Gy=1 J kg$^{-1}$). Dosimetry was based on $N_2O$-saturated solutions containing $10^{-2}$ M KSCN, taking a radiation chemical yield of $G$=0.635 μmol J$^{-1}$ and a molar absorption coefficient of 7580 M$^{-1}$ cm$^{-1}$ at 472 nm for the $(SCN)_2^{\bullet -}$ radical (Schuler et al., 1980). Experiments were performed with a continuous flow of sample solutions at room temperature (~23 °C).