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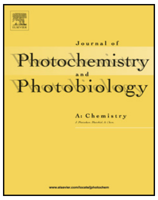

Effect of the spacer length and nitroxide sterical shielding upon photostability of spin-labeled kynurenines

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ABSTRACT

The synthesis of the natural molecular UV filters – kynurenines (KN) – covalently bound to nitroxides has recently been proposed as an alternative for traditional combination of antioxidants and UV-filters in sunscreen formulations. Due to the spin-exchange interaction between KN and nitroxide moieties, the triplet lifetimes in such conjugates are much shorter than in KN molecule, but the triplet quantum yields are significantly higher. The reaction of intramolecular electron transfer between photoexcited KN and nitroxide moieties is the main factor determining the quantum yield of KN-RNO$^\bullet$ conjugates photodecomposition. Searching for a method to suppress this side process we synthesized analogues of previously prepared conjugates with the enlarged spacer between the nitroxide and kynurenine parts and with the sterically hindered access to the nitroxide group. The new spin-labeled kynurenines showed at least three-fold higher photostability compared to the previously synthesized compounds of this type.

1. Introduction

Nitroxides are known to be strong antioxidants, their activity may exceed that of natural antioxidants in some biological models [1]. It has been demonstrated that nitroxides can effectively protect organs against radiation [2–7], inhibit photoinduced oxidation of DNA, proteins and lipids [8–11], and reduce oxidative damage induced by exposure to hydrogen peroxide [12]. Nitroxides are also able to quench the excited singlet and triplet states of molecules [13–16]. Typically, these reactions are not accompanied by the formation of new diamagnetic or paramagnetic reaction products, and results in the restoration of the parent compounds in the ground state [13,16]. The quenching efficacy increases if a nitroxide is covalently bound to the UV absorber [17,18]. It has been demonstrated [19–22] that the major mechanism of the excited state quenching in chromophore-radical coupled systems is the enhanced intersystem crossing induced by the interaction between the photoexcited chromophore and radical species. In some cases, the acceleration of decay of the excited singlet and triplet states may exceed the factor of 1000 as compared to diamagnetic analogues, thereby enhancing the photostability of compounds due to the radical attachment [22,23]. Such hybrid molecules designed on the basis of well-known UV-B absorber ethylhexyl methoxycinnamate (OMC) have been proposed for the complex protection of skin against solar irradiation [24,25].

Modern pharmacology and cosmetology offer a wide range of sunscreens for skin protection against the excessive solar radiation. The main active components of these compositions are chromophoric organic molecules effectively absorbing UV radiation in the range from 280 to 400 nm. The major requirements for these compounds are: high absorption in the UV-A and UV-B regions, high photostability, and low toxicity and phototoxicity. The electronically excited states produced under solar irradiation may react with oxygen yielding reactive oxygen species which are known to produce toxic effects damaging cellular membranes, plasma, mitochondrial and endomembrane systems, and initiating a process of lipid peroxidation. It was demonstrated that the presence of radical traps in sunscreen formulations may prevent the reactive oxygen species formation and thus give an appreciable

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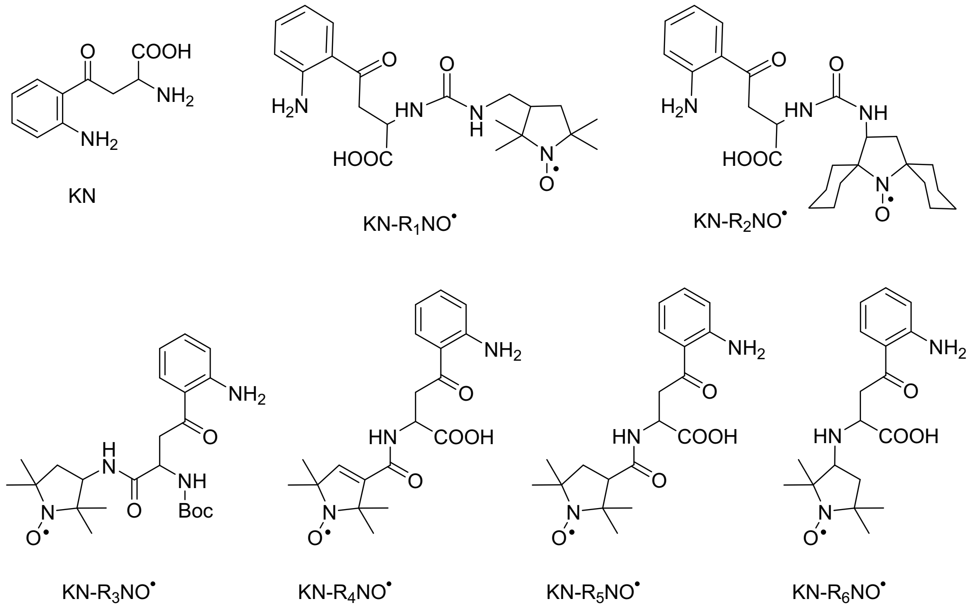


Chart 1. Chemical structures of kynurenine and spin-labeled kynurenines.

positive effect in the terms of slowing the skin aging and reducing the skin photodamage caused by prolonged sun exposure [26–29]. Development of new sunscreen formulations and photostability evaluation of their components is a subject of numerous recent publications, e.g. [30–34].

Recently we have prepared a set of spin-labeled kynurenines (KN-R$_3$NO$^\bullet$, KN-R$_4$NO$^\bullet$, KN-R$_5$NO$^\bullet$, and KN-R$_6$NO$^\bullet$, see Chart 1) [35]. Kynurenine (KN) – a natural amino acid – is a product of tryptophan metabolic transformation, absorbing light in the UV-A region. KN and its derivatives are the native UV filters of the human lens, protecting the retina and the lens itself from photoinduced damages [36–40]. Extensive study of the conjugates revealed efficient quenching of the excited triplet states. However, spin labeling of KN resulted in two undesirable consequences. First, the conjugates gave higher quantum yields of the triplet state compared to KN, which is in agreement with the literature data on spin-labeled chromophores [18,41,42]. Another consequence was the decreased photostability of conjugates; it has been demonstrated that the major factor determining the photostability of KN-RNO$^\bullet$ conjugates is the intramolecular electron transfer between photoexcited KN and nitroxide moieties with formation of the short-lived radicals [35]. Noteworthy, the free radicals formation was not observed in the intermolecular quenching of the triplet state by nitroxides [13,16].

KN-RNO$^\bullet$ conjugate with the shortest spacer between KN and nitroxide showed the fastest quenching of the triplet state and the poorest photostability. Thus, the KN-RNO$^\bullet$ conjugates represent a convenient model to investigate the effects of nitroxide structure and spacer length upon the triplet quantum yield, triplet lifetime and contribution of processes leading to the free radical formation.

In the present work, we report a synthesis of two new KN-RNO$^\bullet$ conjugates, one with the sterically shielded nitroxide moiety and one with the elongated spacer. The study of the photochemical properties of new derivatives revealed that the distancing and/or shielding of the nitroxide moiety noticeably reduce the rate of the intramolecular electron transfer reaction between the photoexcited KN and nitroxide moieties.

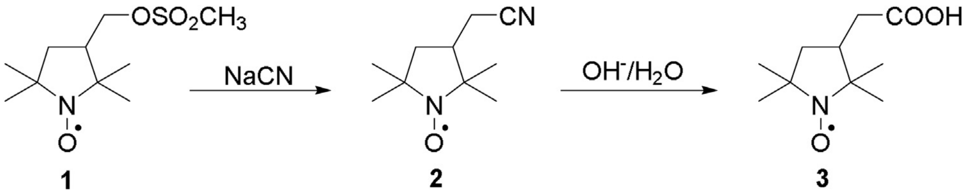
2. Experimental

2.1. Materials

D,L-Kynurenine (Fluka, Germany) and organic solvents (Cryochrom, Russia) were used without additional purification. Double-distilled water and sodium phosphate (reagent grade) were used for the buffer preparation. 3-(((Methylsulfonyl) oxy) methyl)-2,2,5,5-tetramethylpyrrolidin-1-oxyl **1** was prepared according to the literature procedure [43] and used for the synthesis of compounds **2** and **3** (Scheme 1). Sterically shielded 14-carboxy-7-azadispiro[5.1.5.2]pentadecan-7-oxyl **4** was synthesized as described in [44]. *N*-Hydroxysuccinimide esters **5a,b** were synthesized by the standard method of preparation [45] and converted to corresponding isocyanate spin labels **6a,b** (Scheme 2).

2.2. Synthetic procedures and characterizations

3-((3-(3-(2-Aminophenyl)-1-carboxy-3-oxopropyl) ureido) methyl)-2,2,5,5-tetramethylpyrrolidin-1-oxyl (KN-R$_1$NO$^\bullet$) and 14-(3-(3-(2-aminophenyl)-1-carboxy-3-oxopropyl) ureido)-7-



Scheme 1. Synthesis of carboxylic acid **3**.