Solar one-way photoisomerisation of 5′,8-cyclo-2′-deoxyadenosine

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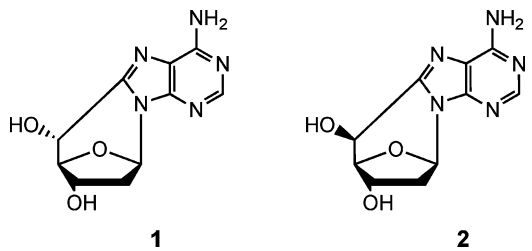
Under sunlight irradiation (5′*S*)-5′,8-cyclo-2′-deoxyadenosine **2** photoisomerises to the (5′*R*) isomer **1**, which is the more easily repaired damage when these cyclopurine lesions are formed in DNA.

Introduction

5′,8-Cyclo-2′-deoxyadenosine (cdA) lesions, which do not result in cleavage of the DNA polymer, are observed among the decomposition products of DNA when it is exposed to ionising radiation and are generated in two diastereomeric forms, depending on the configuration at the C5′ position, *i.e.*, (5′*R*)-isomer **1** and (5′*S*)-isomer **2** (Scheme 1).[1,2] Their mechanism of formation is thought to involve an initial hydrogen abstraction by a hydroxyl radical from the C5′ position of the sugar. This is followed by cyclisation of the C5′ radical onto the C8 position of the base and finally oxidation of the resulting aminyl radical. The overall result is the formation of a new covalent bond between the sugar moiety and the purine base.[3,4]



Scheme 1 Structures of (5′*R*)-cdA **1** and (5′*S*)-cdA **2** lesions.

The (5′*S*)-cdA isomer has been identified in mammalian cellular DNA *in vivo,* and its level is enhanced by conditions of oxidative stress.[5] This moiety can be analysed after complete release from DNA by enzymic hydrolysis, and its level in tissue DNA is comparable to those of other oxidatively induced DNA lesions.[1] For instance, it has been found that (5′*S*)-cdA levels in cellular DNA are similar to those of 8-hydroxy-2′-deoxyguanosine, and three times higher than those of 8-hydroxy-2′-deoxyadenosine.[1] Moreover, (5′*S*)-cdA has been shown to block DNA and RNA polymerases *in vitro* and to be a transcription blocking lesion.[6,7] The (5′*R*)-cdA isomer has also been identified in mammalian cellular DNA *in vivo*, although its background levels are lower than those of (5′*S*)-cdA.[8] Neither of the two diastereomers are repaired *via* recognition by human DNA glycosylases active in the base excision repair (BER) pathway. However, they are substrates (albeit relatively poor ones) for the nucleotide excision repair (NER) mechanism, the (5′*R*)-isomer being repaired more efficiently than its (5′*S*)-counterpart. It has been suggested that, in contrast to several other types of oxidatively generated DNA damage, these cyclonucleosides are chemically stable and can accumulate in DNA at a slow rate over years.[6,7] In some genetic diseases such as xeroderma pigmentosum, where the NER system is defective, the formation of cyclopurines appears to be responsible for neurodegeneration and for the increased risk of certain types of cancer.[5–7]

In view of their biological significance, the aim of the present work has been to investigate the photostability of cdAs, under conditions typical of sunlight irradiation. Moreover, a possible photoisomerisation process would be relevant in connection with the efficiency of their repair.

Results and discussion

Compounds **1** and **2** were obtained in an expedient one-pot procedure by the UV photolysis of 8-bromo-2′-deoxyadenosine in acetonitrile, with a conversion of 65% and in a diastereoisomeric ratio of **1** : **2** = 1.7.[4]

The two isomers were separately irradiated in aqueous solutions, at neutral pH, under both aerobic and anaerobic conditions, using a solar simulator as the light source. The crude reaction mixtures were analysed by HPLC coupled with UV detection, using authentic samples as reference compounds for identification of the products. No photoreaction was observed starting from **1** after 35 h of exposure; by contrast, compound **2** was progressively converted into its isomer **1**. Fig. 1A shows the 3D HPLC plot for the kinetic behaviour of the photolysis of **2**: a build up of **1** coupled with the disappearance of the starting material was clearly observed.

Thus, sunlight irradiation produces a one-way photoisomerisation of **2** to **1**, whereas the latter compound does not isomerise under the same conditions. It should be noted that, unlike pyrimidines, purine DNA bases rarely exhibit direct solar photoreactivity, which requires light absorption in the UVB region. This prerequisite is fulfilled by compound **2**, whose UV-spectrum is shown as an inset in Fig. 1B. As a matter of fact, a clean photoisomerisation of **2** to **1** was also observed upon monochromatic irradiation at $\lambda = 295$ nm (Fig. 1B). The efficiency of the process was compared to the formation of cyclobutane thymine dimers, a well-studied lesion in DNA, using isoabsorptive solutions (A = 0.1) of **1**, **2**, thymidine and thymidylyl-(3′-5′)-thymidine, at the same excitation wavelength. Operating in this

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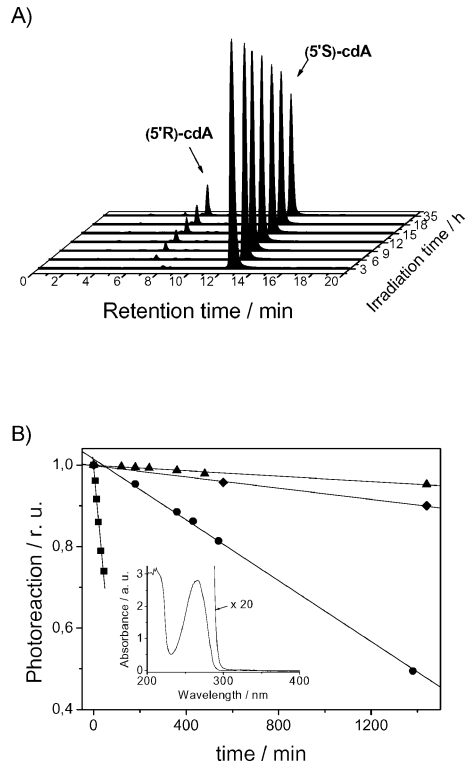


Fig. 1 A: 3D HPLC plot of (5′*S*)-cdA irradiation with a solar simulator. B: photodegradation of **1** (♦), **2** (●), thymidine (▲) and thymidylyl-(3′-5′)-thymidine (■) under N_2, at 295 nm, using isoabsorptive solutions (A = 0.1) of the four compounds at this wavelength. Inset: UV-absorption spectrum of **2** in aqueous solution (0.19 mM).

way, it was found that photoisomerisation of (5′*S*)-cdA was markedly faster than thymidine dimerisation but slower than the equivalent intramolecular process in the dinucleotide. Using phenylglyoxilic acid as an actinometer,[9] the upper limit for the quantum yield of the process was found to be 0.01.

To gain some insight into the photochemical mechanism involved, a laser flash photolysis study was performed. Photolysis of **1** and **2** in an aqueous medium with 266 or 308 nm laser light, under an anaerobic atmosphere, did not result in the formation of any observable transient. However, when the cyclonucleosides were irradiated in the presence of acetone, as a triplet photosensitiser, a new signal was observed (Fig. 2). Thus, the excited triplet state of **1** or **2** is not reached by direct photolysis, but it can be produced by triplet–triplet energy transfer from acetone, which is known to possess a high triplet energy level.[10] The inset of Fig. 2 shows the photosensitised triplet formation ($\tau \sim 0.5$ μs) and decay in water, which was very similar for **1** and **2** ($\tau \sim 9.6$ and 8.2 μs, respectively). Triplet assignment was confirmed by oxygen quenching, which occurred with a rate constant close to the diffusional limit. Hence, formation of **1** upon direct steady-state irradiation must occur from the singlet excited state, as no triplet–triplet absorption was detected in the unsensitised laser flash photolysis experiment. However, this does not allow a possible photoisomerisation following the triplet pathway to be ruled out. In fact, when **2** was subjected to irradiation using longer-wavelength monochromatic light (310 nm), in the presence of acetone, it was also converted into **1** (*ca.* 30% after 24 h); by

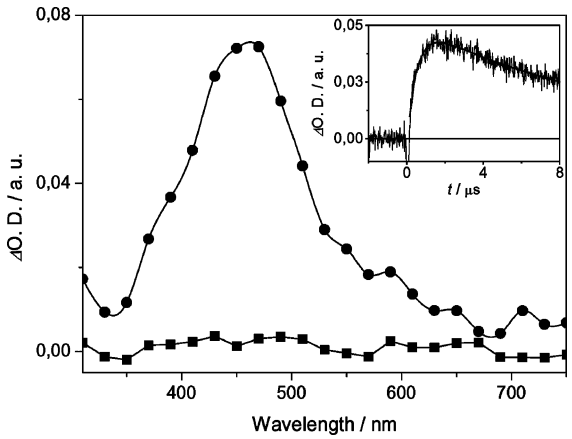
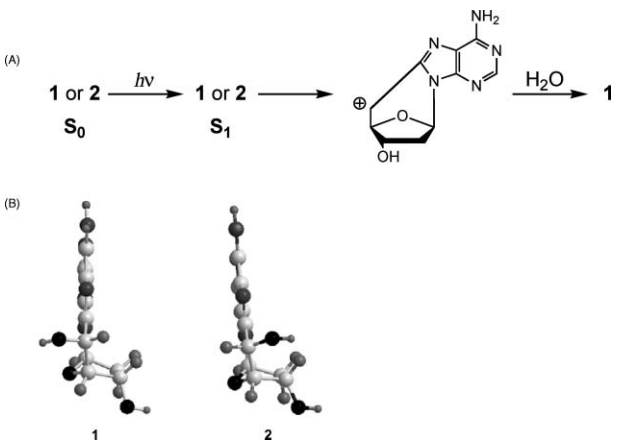


Fig. 2 Transient absorption spectrum obtained from the laser flash photolysis (308 nm) of N_2-purged aqueous solutions containing **1** or **2** (1 mM) in the absence (–■–) and in the presence (–●–) of acetone (0.54 M), taken 0.75 μs after the laser pulse. Inset: time dependence of the T–T absorption of **2** at 450 nm, in the acetone-photosensitised experiment.

contrast, no significant photoisomerisation of **1** to **2** occurred under the same conditions.

The observed photobehaviour of **1** and **2** can be explained by the general mechanism accepted for the photolysis of benzylic alcohols and, in a more general way, for the photocleavage of benzyl–heteroatom σ bonds (see Scheme 2A).[11] It must proceed through a heterolytic cleavage of the C–O bond leading to benzyl-type cations, which subsequently undergo nucleophilic trapping by the solvent with concomitant isomerisation. This is supported by the incorporation of methanol ($MH^+ = 264$), which was observed when the photoreaction was performed in this solvent.



Scheme 2 A: mechanism proposed for the photoisomerisation of 5′,8-cyclo-2′-deoxyadenosines **1** and **2**. B: molecular models of both isomers.

Heterolysis of the C–O bond requires *ca.* 60 kcal mol^{-1} to be possible in energetic terms. This value seems to be lower than both the singlet and the triplet excited state energies of cdAs. The singlet energy of **1** and **2** is unknown, as all attempts to record reliable fluorescence spectra were unsuccessful. However, it clearly has to be higher than the required 60 kcal mol^{-1}, since according to the energy transfer experiments the lower-lying triplet state must be

below that of acetone (79 kcal mol^{-1}) and therefore similar to those reported for related adenosine derivatives (*ca.* 73 kcal mol^{-1}).[10]

Finally, the differences observed between the photobehaviour of the two isomeric cdA lesions must be related to the configuration of the 5′ carbon atom. In this context, the main differentiating feature found in simple MOPAC molecular models (see Scheme 2B) is the closer arrangement between the 5′-OH group and the oxygen atom of the tetrahydrofuran ring of the sugar in the (5′*R*)-isomer. This could facilitate nucleophilic attack by water at C5′ of the benzylic-type cation from its less hindered side, probably assisted by hydrogen bonding between the nucleophile and the ether oxygen.

Conclusions

The observed photoisomerisation of (5′*S*)-cdA to (5′*R*)-cdA might be important due to its potential biological significance, considering the different efficiencies reported for the repair of the two diastereomers by enzymes.[7] When these lesions are formed in DNA under conditions of oxidative stress, it might be presumed that photoisomerisation by sunlight could possibly be used to obtain the more easily repaired lesion. However, it has to be taken into account that the experiments reported here have been conducted with the isolated cyclonucleosides. In order to investigate whether the observed process also occurs under natural conditions, (5′*S*)-cdA should be incorporated into DNA or duplex oligonucleotides and then exposed to UVB-radiation. This is clearly beyond the scope of the present work and constitutes an exciting challenge for future research.

Experimental

Synthesis

Diastereomers (5′*S*)-cdA and (5′*R*)-cdA were obtained in an expedient one-pot procedure by UV photolysis of 8-bromo-2′-deoxyadenosine, and isolated as previously described.[4]

Steady-state irradiation experiments

Broad band irradiations of **1** or **2** (*ca.* 1 mM) were carried out using an Oriel Class A 91192A solar simulator (Stratford, CT, USA) with a 1000 W Xe arc (see Fig. 3 for comparison between the real solar emission spectrum and that of the solar simulator). Its output was adequately filtered to produce a spectrum approximating natural sunlight (1.5 G air mass filter). The spectral output was measured using a grating UV spectroradiometer (Luzchem SPR-01).

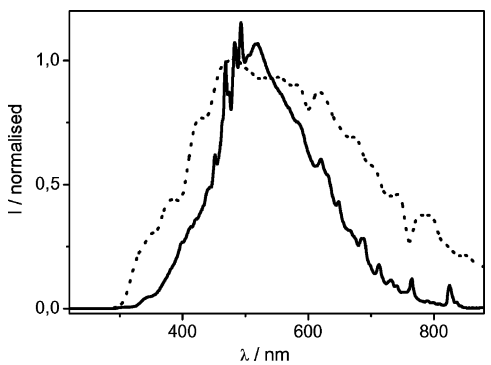


Fig. 3 Solar simulator (–) and real solar (· · ·) emission spectra.

For irradiations at 295 nm or 310 nm the light source was the 75 W Xe lamp of a Photon Technology International Inc. (PTI) LPS-220B spectrofluorometer equipped with an 814 photomultiplier detection system from PTI. The samples were placed inside quartz cuvettes (optical path length 1 cm), and the absorbance was kept at ≈ 0.1 for **1**, **2**, thymidine, thymidylyl-(3′-5′)-thymidine, and phenylglyoxilic acid. In the case of the photosensitised experiment only absorption by acetone was present (A ≈ 0.1). All the photolysis experiments were performed at room temperature (22 °C), under a controlled atmosphere, using aqueous solutions of water purified through a Millipore Milli-RO plus 30 system.

Instrumental analysis

The irradiated mixtures were analysed by reverse-phase HPLC, using a Waters apparatus equipped with an Agilent column (SB-Zorbax, 150 × 4.6 mm, 5 μm) and a Waters 2996 photodiode array detector fixed at a wavelength of 254 nm. For analytical determination, acetonitrile and water were used as the eluents in a 5 : 95 (v/v) ratio at an initial flow rate of 0.4 ml min^{-1} (for 10 min) and subsequently increasing this rate to 0.7 ml min^{-1}, until a total elution time of 25 min had passed. Mass balance was *ca.* 100%, as assessed by the use of adequate standards for calibration.

Time-resolved laser flash photolysis

Nanosecond laser flash photolysis experiments were made with an excimer laser (Xe–HCl–Ne) exciting at 308 nm. The single pulses were *ca.* 17 ns in duration, and the energy was 200 mJ output at the source. A pulsed Oriel-Lo255 Xe lamp was employed as the detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, an Oriel-77200 monochromator, and an Oriel photomultiplier-tube (PMT) system made up of a 77348 side-on PMT tube, a 70680 PMT housing, and a 70705 PMT power supply. The output signal from the oscilloscope (Tektronix TDS-640A) was transferred to a personal computer. Concentrations of **1** and **2** were *ca.* 1 mM, whereas that of the photosensitiser (acetone) was 0.54 M. The absorbance was *ca.* 0.30 in the laser cell at the excitation wavelength.

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