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DNA CLEAVAGE BY PHOTOEXCITED DIAZOARENES[§]

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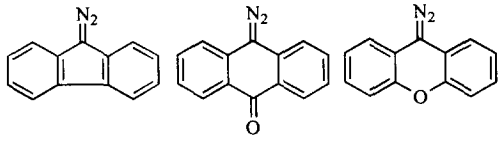
Abstract: Photolysis of simple, readily available arene diazo compounds leads to the cleavage of DNA.

Considerable attention is currently being focused on the design of artificial nucleases for use in numerous biochemical and biomedical applications. While most of the hitherto reported DNA-cleaving agents have been activated thermally, in recent years, there is an increasing emphasis on photoactivated cleavage agents, because this methodology possesses significant practical advantages. In particular, photonucleases can be triggered by exposure to light; light is an attractive 'co-factor' since it is easy to manipulate. Thus, a plethora of photochemically reactive molecules including quinones, napthalimides, chlorobithiazoles, triazoles, thiopyridones, porphyrins and metal complexes, amongst others, are currently being scrutinized for their photonuclease activity.[1] Photogenerated ion, radical or oxygen-centered reactive species have been proposed to be responsible for cleaving DNA in a majority of these cases. However, carbenes, which are known to be generated upon photoirradiation of diazo compounds and to be reactive towards a wide variety of organic and bioorganic substrates[2] including proteins,[3] have rarely been tested for their ability to cleave DNA. Some studies have been reported on DNA photocleavage brought about by arene diazonium salts[4] and also α-diazo ketones[5].This is all the more surprising considering the fact that the naturally occuring anti-tumor agents kinamycin and prekinamycin, have the diazo group present in them. Herein, we demonstrate that carbenes generated upon photoirradiation of simple, readily available arene diazo compounds are capable of cleaving DNA.



1 **2** **3**

[§]Dedicated to Professor Harold Shechter on the occasion of his 75th birthday

9-Diazofluorene (**1**), 9-diazoanthrone (**2**) and 9-diazoxanthene (**3**) were chosen for this purpose not only because of their ready availability and well-known photochemical reactivity[6] but also for their planar, aromatic structures[7] which are desirable features for binding with DNA. These diazo compounds were freshly prepared just prior to experiments with DNA either by the reaction of HgO/AgO and ethanolic KOH with the corresponding hydrazones in diethyl ether (**1** and **3**) or by the diazo transfer reaction using tosyl azide (**2**).[8] They showed the expected UV-VIS spectra in DMF with the peak maxima of the longest wavelength bands appearing at 348, 431 and 344 nm for **1**, **2** and **3**, respectively. Addition of increasing amounts of calf- thymus DNA (1 - 200 μM, buffer A: 5mM tris, 50mM NaCl, pH 8.0) to solutions containing **1**, **2** or **3** (≈ 20 μM, buffer A, 2 - 3% DMF) resulted in hypochromism (20- 45%) and bathochromic shifts (1 - 3 nm) of their peak maxima and also in the appearance of isosbestic points in the corresponding spectra suggesting DNA-binding. The intrinsic binding constants as obtained from the absorption titration method are as high as 1 - 3 x 10^4 M^{-1} (±10%). All these observations are reminiscent of those reported earlier for DNA-intercalation by various planar, aromatic molecules[9] and argue in favour of a similar intercalative mode of binding by **1**, **2** and **3** with DNA.

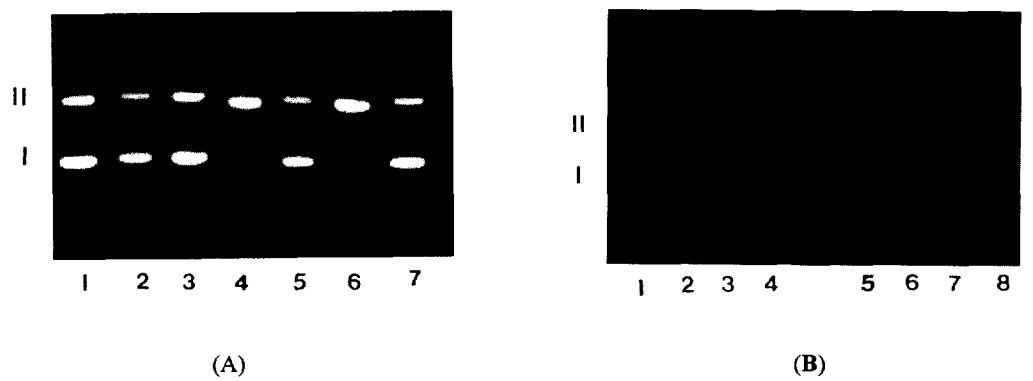


Fig. 1. *Light induced nuclease activity of diazo compounds 1, 2 and 3 (100 μM) with pBR 322 DNA (100 μM in nucleotides):* (**A**)*'Dark' and 'Light' (30 min., 350 ± 5 nm for 1 and 3, 430 ± 5 nm for 2) experiments:* Lanes 1, 2 and 3: DNA + **2** (45), **1** (49) or **3** (47) in dark. Lanes 4, 5 and 6, DNA + **2** (70), **1** (45) or **3** (73) upon irradiation, lane 7 (pBR 322 DNA dark control) (45): (**B**) *Results of 'inhibition' studies carried out with 2 and 3, 'Light' Experments:* Lanes 1- 3: (DNA + **2**) + 2-propanol (38), PPh_3 (32) or cumene (31), respectively, Lane 4: DNA + **2** (60), Lanes 5 - 7: (DNA + **3**) + 2-propanol (52), PPh_3 (51) and cumene (51), respectively, Lane 8: DNA + **3**(70). Numbers in the paranthesis refer to % of Form II DNA.

Photoirradiation (λ_{ex}: 350 ± 5 nm for **1** and **3**, 430 ± 5 nm for **2**) experiments probing the reactivity of these diazo compounds (10 - 100 μM) with the duplex were carried out using supercoiled pBR 322 DNA (100 μM nucleotides) and the reaction was followed by the agarose gel electrophoresis method as described earlier.[1g,h, 10] Control runs showed that untreated DNA does not undergo any cleavage in the dark and even upon irradiation by a 350 (or 450) nm light for 2h. Similarly, DNA nicking was not observed for pBR 322 treated with each of the diazo compounds in the dark (Lanes 1-3, Fig. 1) and only for compound **1** upon

irradiation for 30 min (Lane 5). On the other hand, irradiation of DNA in the presence of compounds **2** and **3**, caused generation of the circular form (form II) as shown in Lanes 4 and 6 of this figure. Thus, under identical experimental conditions, the DNA-nicking efficiencies follow an order: **3** > **2** > **1**. Accordingly, increasing the irradiation time to 60 min. resulted in further significant relaxation of the supercoiled DNA only for **2** and **3**, with **3** being able to completely convert the supercoiled form to the circular form.

Effects due to 2-propanol, triphenylphosphine (PPh_3) and cumene on the observed photonuclease activity of **2** and **3** are illustrated in Fig. 1(b). Results obtained upon photoirradiation of these two reactive compounds in the presence of DNA and various 'inhibitors' including the above three are summarized in Fig. 2. While D_2O, mannitol, DMSO and SOD do not affect the DNA-nicking efficiencies of these compounds, there is a moderate-to-strong inhibition by DABCO, 2-propanol, PPh_3 and cumene. The inability of D_2O to enhance the DNA-nicking effiency can be taken as an evidence to show that $^1O_2$ is not involved in the reaction. That neither OH nor $O_2^{-\cdot}$ play a role in the cleavage mechanism is established by the lack of inhibition of DNA cleavage in the presence of mannitol, DMSO and SOD. Thus, we reason that inhibiton exhibited by the other added reagents collectively provide evidence for the participation of carbenes in the observed DNA cleavage by the two reactive diazo compounds. Indeed, reaction of carbenes with PPh_3 is known to be fast, yielding ylides.[2] Similarly, reaction of carbenes with 2-propanol and cumene is also known to be efficient.[2,6b]

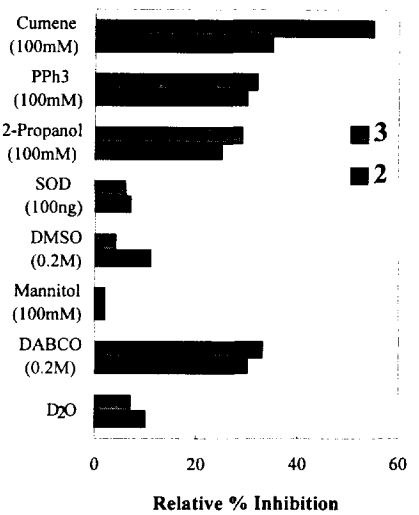


Fig. 2. Effect of various 'inhibitors' on the light-induced neclease activity of **2** and **3** (Error limits ±5%).

Generally, DABCO is employed for probing the participation of $^1O_2$ in the DNA cleavage experiments. However, carbenes are also capable of reacting with amines, including tertiary amines, at their nitrogen centres.[2] This fact, together with the observed inability of D_2O to enhance the DNA photocleavage of **2** and **3**, suggests that the inhibitory effect exhibited by DABCO here can be rationalized in terms of its reactivity with carbenes and not with $^1O_2$. By the same token, it is not unreasonable to expect that carbenes are

capable of alkylating the nucleobases on the DNA and initiate the cleavage. An additional mechanism which involves hydrogen abstraction by the carbene as the key step cannot be ruled out.

In summary, this study demonstrates that carbenes obtained upon visible light irradiation of simple, readily available diazo compounds are effective in cleaving DNA. Encouraged by this result, we are currently probing the intimate mechanism of DNA cleavage by these and other carbene species derived from water-soluble, red-light absorbing diazo compounds.

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10. For the gel electrophoresis experiments, supercoiled pBR 322 DNA (100 μM nucleotides; buffer B: 10 mM Tris, pH 8.0) was treated with an 100 μM (buffer B: 2 - 3% DMF) of the diazo compound (and the 'inhibitor' in cases where applicable) and the mixture was incubated for 1h in the dark. Irradiation experiments were carried out by keeping these pre-incubated samples inside the sample chamber of a JASCO Model FP-777 spectrofluorimeter. The samples were analyzed by 0.8% agarose gel electrophoresis (tris-acetic acid- EDTA buffer, pH = 8.0) at 40 V for 5h. The gel was stained with 1μg/ml ethidium bromide for 0.5 h. after which it was analyzed using the UVP gel documentation system GDS 2000 and was also directly photographed and developed as described previously.[1g,h]

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