PHOTOCHEMISTRY OF N^6-METHOXYADENOSINE AND OF N^4-HYDROXYCYTIDINE AND ITS METHYL DERIVATIVES II: PHOTOINDUCED RUPTURE OF THE N—O BOND

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(Received March 10, 1982)

Summary

The UV irradiation of dilute aqueous and ethanolic solutions of N^4-hydroxycytidine and its $N_{(3)}$-, N^4- and N^4-*O*-methyl derivatives and of N^6-methoxyadenosine leads to rupture of the N—O bond and the formation of cytidine and its methyl derivatives or adenosine. The reaction proceeds via the homolytic dissociation of the N—O bond in the triplet state followed by interaction of the resultant radicals with the solvent. The path of the reaction is not affected by the pH of the solution or the wavelength of the exciting light (254 - 313 nm). The quantum yields of the N—O bond cleavage were determined for various ionic and tautomeric forms of N^6-methoxyadenosine and of N^4-hydroxycytidine and its methyl derivatives.

1. Introduction

The photochemical behaviour of many analogues and derivatives of nucleic acid components such as 4-thiouridine [1 - 3], 5-bromouridine [4], the azapyrimidines [5 - 7], $N_{(1)}$-hydroxyadenine and $N_{(1)}$-methoxyadenine [8 - 11] is quite uncommon. An understanding of the mechanisms of the photoinduced reactions of these compounds will enable the photochemical approach to be applied successfully to structural and functional studies of nucleic acid and nucleoproteins (*e.g.* ref. 4).

The UV irradiation (λ = 254 nm) of N^6-methoxyadenosine, N^4-hydroxycytidine and N^4-methoxycytidine has been shown to produce an unusual photochemical reaction — the rupture of the N—O bond with the formation of adenosine or cytidine [12]. This reaction is of interest for the following reasons. Firstly the available data indicate that hydroxylamine- and *O*-methylhydroxylamine-induced mutations [13] are caused by the for-

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mation of groups of this type. Selective demodification may give direct experimental evidence for this proposition. Secondly N^4-hydroxycytidine, N^4-methoxycytidine and N^6-methoxyadenosine residues imitate normal nucleic acid components in some enzymatic systems [14, 15]. This suggests that polynucleotides containing normal and hydroxylamine-modified residues may form similar nucleoprotein complexes. The photoinduced rupture of the N—O bond probably proceeds via homolytic dissociation. The radicals produced in this way have an unpaired electron on the exocyclic nitrogen (denoted by $\dot{N}$) and may attack adjacent amino acid residues of the protein to form a new type of polynucleotide–protein cross-linking. The production of such UV-induced cross-linking provides a new approach to the investigation of the structure of nucleoprotein complexes [16, 17].

A detailed study of the photoinduced cleavage of the N—O bond in the monomers N^4-hydroxycytidine, N^4-methoxycytidine and N^6-methoxyadenosine is presented in this paper.

2. Materials and methods

Cytidine and adenosine (Reanal, Hungary) were used without additional purification. Hydroxylamine hydrochloride and *O*-methylhydroxylamine hydrochloride were recrystallized from water. The *N*-methylhydroxylamine oxalate was donated by Dr. R. M. Khomutov (Institute of Molecular Biology, U.S.S.R. Academy of Sciences). *O*,*N*-dimethylhydroxylamine hydrochloride was obtained from benzhydroxamic acid by permethylation [18] followed by hydrolysis [19]. 4-thiouridine was synthesized from uridine according to the procedure described in ref. 13, and $N_{(3)}$-methylcytidine (Fig. 1, X) was prepared by the methylation of cytidine [20].

The preparation of N^6-methoxyadenosine (I) by reacting *O*-methylhydroxylamine with adenosine, the preparation of N^4-hydroxycytidine and N^4-methoxycytidine (IIIa and IIIb) and the purification of these compounds were performed as described earlier [21].

N^4-methylcytidine (VII) was prepared as follows. 130 mg (0.5 mM) of 4-thiouridine in 5 ml of a 1 M solution of methylamine hydrochloride (pH 5.0) was kept at 50 °C for 4 h. 10 ml of ethanol was added, the precipitate was filtered and removed, the filtrate was evaporated and the product was purified by thin layer chromatography (TLC) using system 1 (Table 1). The yield of the chromatographically and spectrally pure product was 112 mg (87%).

N^4-methyl-N^4-hydroxycytidine (VIa) was prepared as follows. 103 mg (0.4 mM) of 4-thiouridine in 4 ml of a 4 mM solution of *N*-methylhydroxylamine oxalate was kept at 50 °C for 60 h. The solution was filtered, 10 ml of ethanol was added and the precipitate of *N*-methylhydroxylamine oxalate was removed. The filtrate was evaporated twice with 5 ml of acetone; the pH was adjusted to 7 for each evaporation. The product was purified by TLC using system 1. The yield of chromatographically and spectrally pure VIa was 97 mg (86%).

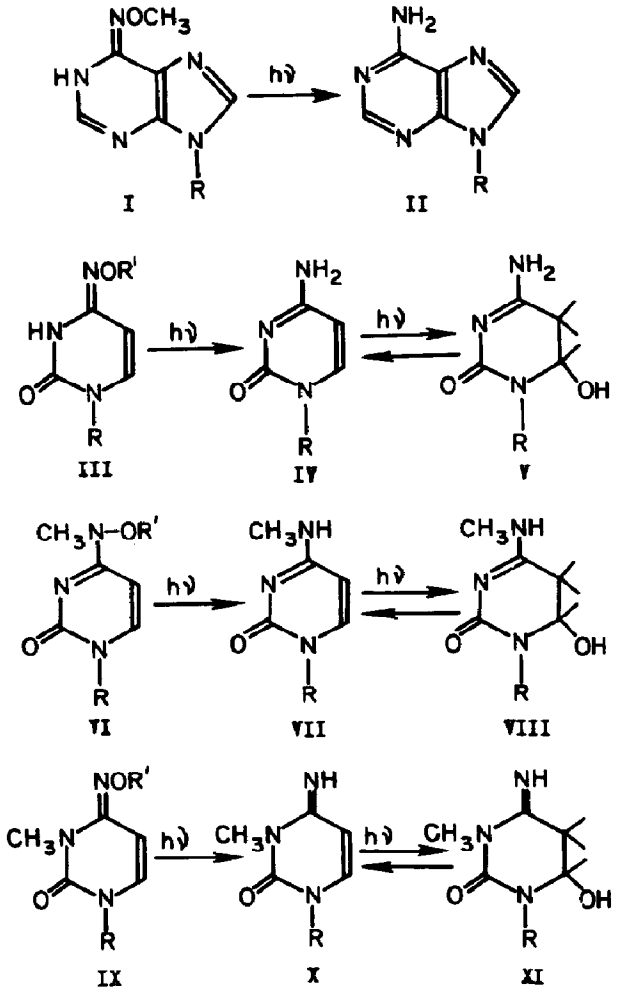


Fig. 1. Transformations of the compounds investigated (R ≡ β-D-ribofuranosyl; a, R′ ≡ H; b, R′ ≡ CH_3).

N^4-methyl-N^4-methoxycytidine (VIb) was prepared as follows. 130 mg (0.5 mM) of 4-thiouridine in 5 ml of a 1 M solution of *O*,*N*-dimethylhydroxylamine (pH 5.2) was kept at 50 °C for 100 h. After filtration, 10 ml of ethanol was added and the *O*,*N*-dimethylhydroxylamine hydrochloride precipitate was removed. The filtrate was evaporated twice with 5 ml of acetone; the pH was adjusted to 7 for each evaporation. The residue was dissolved in 20 ml of 0.5 M acetic acid and the resulting solution was passed through a column (1 cm × 15 cm) containing AG50W × 8 resin (200 - 400 mesh, BioRad, U.S.A.) in H^+ form equilibrated with 0.5 M acetic acid. The column was then washed with 0.5 M acetic acid followed by water to pH 5, and N^4-methyl-N^4-methoxycytidine was eluted with 0.1 M NH_4OH. The yield of chromatographically and spectrally pure N^4-methyl-N^4-methoxycytidine was 131 mg (90%).

TABLE 1

R_f values for nucleosides on cellulose

Nucleoside	*Solvent system*[a]				
	1	*2*	*3*	*4*	*5*
N^6-methoxyadenosine (I)	0.25	0.43	0.76	0.44	0.77
Adenosine (II)	0.17	0.25	0.67	0.29	0.51
Uridine	0.22	0.65	0.57	0.26	0.75
4-thiouridine	0.41	0.82	0.65	0.53	0.76
Cytidine (IV)	0.12	0.48	0.65	0.10	0.81
N^4-hydroxycytidine (IIIa)	0.47	0.42	0.63	0.31	0.80
N^4-methoxycytidine (IIIb)	0.48	0.49	0.84	0.53	0.90
N^4-methyl-N^4-hydroxycytidine (VIa)	0.27	0.46	0.55	0.27	0.93
N^4-methyl-N^4-methoxycytidine (VIb)	0.43	0.62	0.82	0.36	0.89
N^4-methylcytidine (VII)	0.23	0.61	0.75	0.11	0.86
$N_{(3)}$-methyl-N^4-hydroxycytidine (IXa)	0.20	0.64	0.83	0.77	0.75
$N_{(3)}$-methyl-N^4-methoxycytidine (IXb)	0.71	0.76	0.90	0.81	0.90
$N_{(3)}$-methylcytidine (X)	0.08	0.61	0.77	0.10	0.93

[a] 1, 86:14 *n*-butanol:water; 2, 7:2:1 isopropanol:concentrated HCl:water; 3, 7:2:1 isopropanol:concentrated NH_4OH:water; 4, 77:13:10 butanol:water:formic acid; 5, water (pH 5).

$N_{(3)}$-methyl-N^4-hydroxycytidine (IXa) was prepared as follows. 80 mg (0.35 mM) of $N_{(3)}$-methylcytidine in 4 ml of a 1 M solution of hydroxylamine (pH 7.0) was kept at 45 °C for 20 h. The pH was then adjusted to 1 using concentrated HCl and the mixture was incubated for 20 h at 37 °C. A suspension of Dowex AG1 X 8 (200 - 400 mesh) in OH^- form was added to pH 6.0. The resin was filtered and the filtrate was evaporated twice with 10 ml of acetone; the pH was adjusted to 7 for each evaporation. The product was purified by TLC using system 1. The yield of chromatographically and spectrally pure product was 70 mg (74%).

$N_{(3)}$-methyl-N^4-methoxycytidine (IXb) was obtained by a similar method from $N_{(3)}$-methylcytidine and *O*-methylhydroxylamine. The yield of chromatographically and spectrally pure product (IXb) was about 80%.

The purity of all the substances obtained was determined by TLC on cellulose powder (FND, Filtrac, G.D.R.) (see Table 1) and by UV and proton magnetic resonance spectroscopy.

The UV absorption spectra of the ionic and tautomeric forms of the *syn* and *anti* isomers of $N_{(3)}$-methyl-N^4-hydroxycytidine and $N_{(3)}$-methyl-N^4-methoxycytidine (IXa and IXb) and their ratios in aqueous solutions have been presented elsewhere [21]. The ratio of the molar extinction coefficients for the neutral amino and imino tautomers IIIa and IIIb was assumed to be the same as that for the corresponding fixed forms (VIa:IXa and VIb:IXb) [21]. The spectra of cytidine and adenosine were obtained from ref. 22, and those of N^4- and $N_{(3)}$-methylcytidine were obtained from refs. 23 and 24 respectively (Fig. 2). The spectra of N^4- and $N_{(3)}$-methylcytidine

photohydrates, the quantum yields of photohydration and the rate constants of the photohydrate reversions (Table 2) were determined from the changes in the absorption spectra of solutions VIII and XI during both irradiation (λ = 254 nm) and their subsequent storage in the absence of irradiation.

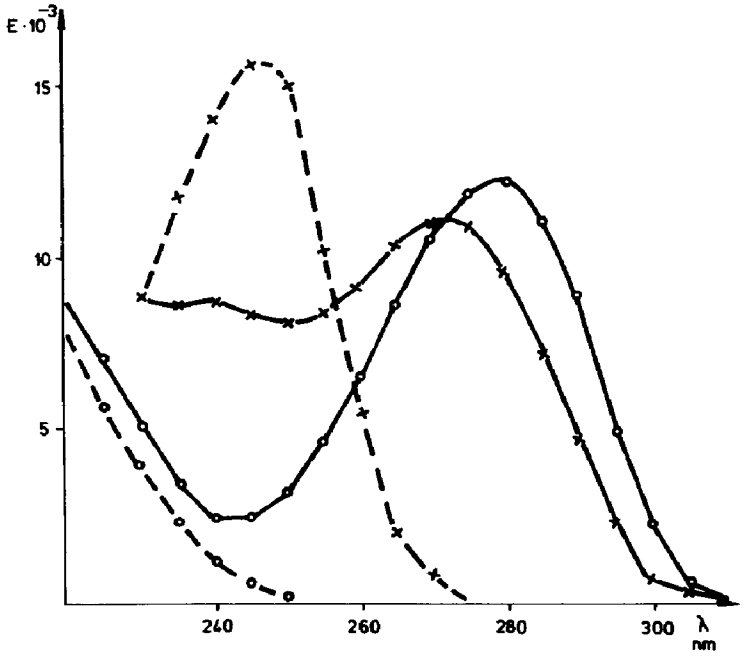


Fig. 2. The spectra of N^4-methylcytidine (VII) (—×—) and $N_{(3)}$-methylcytidine (—○—) and of N^4-methylcytidine photohydrate (VIII) (— —×— —) and $N_{(3)}$-methylcytidine photohydrate (XI) (— —○— —).

TABLE 2

The photohydration quantum yields ϕ for cytidine, $N_{(3)}$-methylcytidine and N^4-methylcytidine and the rate constants K of reversion of the corresponding photohydrates

Nucleoside	$\phi \times 10^3$	k ($\times 10^{-3}$ min^{-1})
Cytidine	9.5 (9.0 [25])	5.8 (4.9 [26])
$N_{(3)}$-methylcytidine	1.9	1.8
N^4-methylcytidine	6.7	0.2

λ = 254 nm; $I_0 = 3 \times 10^{15}$ quanta s^{-1} cm^{-2}; water (pH 6) at 20 °C.

Aqueous or ethanolic solutions ($(1 - 5) \times 10^{-4}$ M) of the compounds under study were irradiated (λ = 254 nm) using a low pressure mercury lamp either in a quartz coaxial reactor [12] or in rectangular quartz cells at 20 °C. Irradiation in the 250 - 300 nm range was performed using a high pressure xenon lamp with a monochromator. The intensity of the light changes from 0.2×10^{15} to 7×10^{15} quanta s^{-1} cm^{-2} when the wavelength is changed from 250 to 300 nm. A high pressure mercury lamp with a BS-4 glass filter 3 mm

thick was used for the irradiation at wavelengths above 300 nm. An additional liquid filter (0.2 g K_2CrO_4 l^{-1} + 1 g Na_2CO_3 l^{-1}) 10 mm thick was used to isolate light of wavelength 313 nm.

The intensity of the incident light in the range 254 - 290 nm was measured by the photohydration of an aqueous solution containing 10^{-4} M uridine (the quantum yield of uridine photohydration was taken as 2.16×10^{-2} [27] and at wavelengths of 290 nm or above was determined by ferrioxalate actinometry [28].

For photosensitized cleavage of the N—O bond 8×10^{-4} M of benzophenone was added to neutral nucleoside solutions (about 10^{-4} M). The solution was then saturated with nitrogen by bubbling it through for 30 min and was irradiated at $\lambda > 300$ nm. A simultaneous irradiation of nucleoside solutions which did not contain benzophenone was performed. The degree of transformation was monitored by TLC on cellulose using system 1 after extracting the benzophenone five times with ether. The substances were eluted from chromatograms with water and the quantity produced was measured spectrophotometrically. In all other cases the reaction pattern was controlled either by TLC of the reaction mixture on cellulose (Table 1), after reversion of cytidine or methylcytidine photohydrates, or by measuring the changes in the spectra of solutions in the range 200 - 300 nm during and after irradiation. The quantum yields for loss of the primary products were determined using the following expression which was obtained as in ref. 12 but without assuming the optical density D of the solution to be much less than unity:

$$\phi = \frac{6.02 \times 10^{20}}{I_0 \epsilon t} \lg\left(\frac{10^{\epsilon C_0 l} - 1}{10^{\epsilon C_t l} - 1}\right) \quad (1)$$

where I_0 (quanta cm^{-2} s^{-1}) is the intensity of the incident light, C_0 and C_t are the concentrations of the substance before and after irradiating for t min, ϵ is the molar extinction coefficient of the substance and l is the optical length.

As can easily be seen from eqn. (1) the C_t values must be determined experimentally to calculate ϕ. These values were determined by a least-squares method from the total absorption spectra $F_t(\lambda)$ of irradiated solutions [29] measured at various time intervals t on the basis of known absorption spectra $f_t(\lambda)$ of the initial, intermediate and final products which are revealed by spectroscopic examination of the photoreaction (these products are noted by i). Thus the C_t values can be calculated from the system of equations by a least-squares method:

$$F_t(\lambda) = \sum_i C_{it} f_i(\lambda) \quad (2)$$

where λ is the wavelength of the spectral interval under investigation. It is assumed in these equations that $f_i(\lambda)$ is time independent.

In the determination of the ϕ value using eqn. (1) it should be remembered that if there is a large overlap between the absorption spectra of

the starting compound and of the primary photoproduct then the starting compound may be shielded by the photoproducts. This shielding effect is not included in expression (1). Therefore ϕ values were determined at low transformations of the starting compound to avoid shielding effects.

In some cases the starting compounds exist in solution as a mixture of ionic and/or tautomeric forms (this has been considered in detail previously [21]). As the quantum yields ϕ_i of phototransformation for some products are independent of the wavelength of the exciting light, if both the dependence of the quantum yield ϕ_i of decomposition of the equilibrium mixture of starting products on the wavelength of irradiation and the number of quanta absorbed by each of the starting compounds (when D is very much less than unity this number is proportional to D_i) are known, the values of ϕ_i can be evaluated from the system of equations

$$\phi_\Sigma(\lambda_j)D_\Sigma(\lambda_j) = \sum_i \phi_i D_i(\lambda_j) \tag{3}$$

where j denotes the wavelength of the absorption spectra. The left-hand side of these equations consists of values proportional to the number of decomposed molecules and the right-hand side is the sum of all the photoproduct molecules.

3. Results and discussion

3.1. The reaction scheme

It has previously been shown that the only primary photoreaction occurring when neutral aqueous solutions of N^6-methoxyadenosine (I), N^4-hydroxycytidine (IIIa) and N^4-methoxycytidine (IIIb) were irradiated at 254 nm was rupture of the N—O bond with the formation of peroxides and adenosine or cytidine [12]. The change in the absorption spectra under UV irradiation and the analysis of irradiated solutions by chromatography show that this bond rupture also occurs for acid and alkaline aqueous solutions (pH 0.3 - 13.5), for neutral ethanol solutions, for fixed tautomers of N^4-hydroxycytidine (VI) and N^4-methoxycytidine (IX) on irradiation in the wavelength interval 254 - 313 nm and for sensitized photoreactions in the presence of benzophenone. Cytidine (IV), N^4-methylcytidine (VII) and $N_{(3)}$-methylcytidine (X) formed in the primary photoreactions from the corresponding N-hydroxy and N-methoxy derivatives transform on irradiation to the photohydrates V, VIII and XI respectively which undergo further spontaneous reversion (Table 2).

Almost complete reversion of the photohydrates of cytidine [25, 26], $N_{(3)}$-methylcytidine and N^4-methylcytidine even after 70% - 80% photohydration has been shown by special experiments. Figure 1 shows the transformations of the compounds investigated. According to the chromatographic and spectral data no other products are formed on irradiation of the N-hydroxy and N-methoxy derivatives of adenosine and cytidine.

TABLE 3

The effect of benzophenone on the photoinduced rupture of the N—O bond in *N*-hydroxy and *N*-methoxy nucleosides

Nucleoside	*Ratio of benzophenone to nucleoside* (mol mol^{-1})	*Irradiation time* (min)	*Degree of transformation* (%)	
			With benzophenone	*Without benzophenone*
N^6-methoxyadenosine (I)	6.9	180	89	18
N^4-methoxycytidine (IIIb)	5.0	180	23	6
N^4-methyl-N^4-hydroxycytidine (VIa)	5.5	60	33	15
N^4-methyl-N^4-methoxycytidine (VIb)	11	10	25	23
$N_{(3)}$-methyl-N^4-methoxycytidine (IXb)	8.4	15	11	3

Benzophenone concentration, 7.88×10^4 M; $\lambda > 300$ nm; $I_0 = 4 \times 10^{15}$ quanta cm^{-2} s^{-1}.

3.2. Reaction mechanism

When I, IIIb and IXb are irradiated with light of wavelength greater than 300 nm in the presence of benzophenone, which is an efficient donor of electronic excitation from its lower triplet state [28], there is a fourfold increase in the rate of N—O bond scission (Table 3). These compounds are present in neutral aqueous solutions mainly or totally in the imino form [21]. In the case of VIa, which is present in solution as an equilibrium mixture of the neutral amino tautomer and the bipolar ion (ratio 3:7) [21], the addition of benzophenone leads to a twofold increase in the reaction rate. However, in the case of VIb (pure amino tautomer) the addition of benzophenone has little effect on the reaction rate. The behaviour of the imino forms can be explained by the efficient transfer of electronic excitation from the lower triplet state of benzophenone to the triplet state of the compounds under investigation. This transfer does not appear to take place in amino tautomers. This may be due to the fact that the triplet energy of the amino tautomer is 0.7 eV higher than that of the imino tautomer [30], *i.e.* the T_1 state of the amino tautomer may be located at a higher energy than that in benzophenone. In the mixture of the neutral amino form and the bipolar ion (VIa) the energy transfer appears to occur only on the bipolar ion.

The quantum yield of the photoreaction does not change when the light intensity increases from 0.45×10^{15} to 4.5×10^{15} quanta cm^{-2} s^{-1}. The absence of such a dependence suggests that the photoreaction under study has a monophotonic character since saturation of the triplet states at room temperature with the light intensities used in this work is unlikely [31].

The quantum yield of photoscission of the N—O bond is almost constant in the pH range where the neutral forms of I, IIIa and IIIb are present (Table 4). This indicates the non-ionic character of the reaction. The formation of peroxides together with adenosine and cytidine on irradiation of

TABLE 4

Total quantum yields of the N—O bond rupture on irradiation of aqueous and ethanolic solutions of N^6-methoxyadenosine, N^4-hydroxycytidine and N^4-methoxycytidine

Nucleoside[a]	$\phi \times 10^3$[b]	
	Water	*Neutral ethanol*
N^6-methoxyadenosine (I) (pK_1 = 3.1; pK_2 = 10.4)	3.0 (pH 1.75) 7.0 (pH 4.70) 8.4 (pH 6.55) 8.6 (pH 8.36) 19.0 (pH 12.5)	13.4
N^4-hydroxycytidine (IIIa) (pK_1 = 2.3; pK_2 = 10.2)	14.5 (pH 0.26) 0.42 (pH 5.10) 0.50 (pH 7.05) 32.0 (pH 13.5)	0.52
N^4-methoxycytidine (IIIb) (pK_1 = 1.0; pK_2 = 11.0)	15.0 (pH 0.26) 1.79 (pH 4.14) 2.0 (pH 6.35) 1.8 (pH 8.20) 14.0 (pH 13.0)	1.9

λ = 254 nm; $I_0 = 3 \times 10^{15}$ quanta cm^{-2} s^{-1}.
[a] The pK values were obtained from previous work [21].
[b] The relative error in the determination of ϕ did not exceed ±17%.

I, IIIa and IIIb (see ref. 12) supports this proposition and is strong evidence in favour of the radical mechanism.

Thus the photoinduced rupture of the N—O bond of the compounds under investigation is a monophotonic reaction which appears to proceed through the lower triplet state by the homolytic dissociation of the N—O bond as shown in Fig. 3. The radicals formed either react with the solvent to form peroxides and adenosine or cytidine or recombine to give the starting compounds, leading to a decrease in the quantum yield of decomposition.

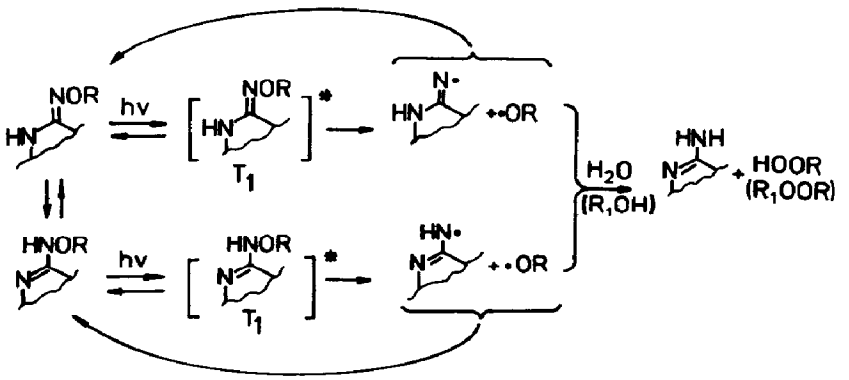


Fig. 3.

3.3. The efficiency of the photoreaction

Since the only primary photoreaction for all the substances investigated is the scission of the N—O bond, a comparison of the rates of this reaction for various compounds under a wide range of conditions allows the effect of the nature of the heterocycle, the presence and position of the methyl groups, the ionic and/or tautomeric state and the *syn* and *anti* location of the N—O groups to be determined.

It should be noted that the quantum yield of N—O bond rupture for a specific structure is independent of the wavelength of the irradiating light. The dependence of the quantum yield on wavelength observed in some cases (Table 5) is due to the presence of an equilibrium mixture of tautomeric and/or ionic forms in the solution. When the spectra of these forms are known their quantum yields can be calculated as described in Section 2.

N^6-methoxyadenosine exists in solution as an equilibrium mixture of amino and imino tautomers in the ratio 7:3 [21]:

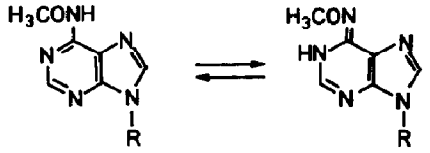


Table 5 shows that the quantum yield of N—O bond scission is two orders of magnitude greater for the neutral amino tautomer than for the corresponding imino tautomer. The quantum yield of N—O bond rupture for the only anionic form of N^6-methoxyadenosine is twice that for the neutral amino tautomer.

The protonation of N^6-methoxyadenosine can afford three types of cation [21] (Fig. 4) but their ratio is unknown. Therefore the effective value of the total quantum yield of the photoreaction is presented in Table 5. The dependence of this value on the irradiating wavelength suggests that the cation whose absorption contribution increases with increasing wavelength possesses the highest reactivity.

The neutral forms of N^4-hydroxycytidine and N^4-methoxycytidine exist in solution as equilibrium mixtures of amino and imino tautomers in the ratios 1:9 and 1:20 respectively [21]. The photocleavage of the N—O bond of these compounds also proceeds more intensively for amino

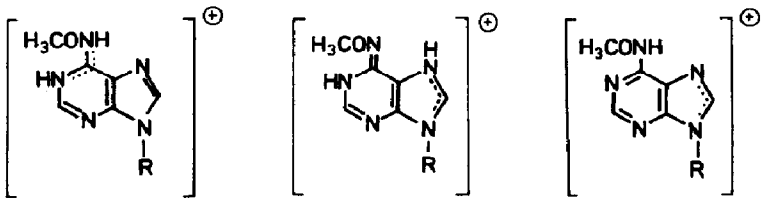


Fig. 4.

TABLE 5

The quantum yields of N—O bond scission for various ionic and tautomeric forms of *N*-hydroxy and *N*-methoxy derivatives of cytidine and adenosine under UV irradiation

Nucleoside	*Ionic form*	*Tautomeric form*[a]	$\phi \times 10^3$[b]	
			At 254 nm	*At 313 nm*
N^6-methoxyadenosine (I)	Cation	Equilibrium mixture of three tautomers	3.1	5.7[c]
	Neutral	Mixture of amino and imino (7:3)	8.6	0.10
		Amino	10.4[d]	—
		Imino	0.10	—
	Anion		19.0	20.0
N^4-hydroxycytidine (IIIa)	Cation		14.5	12.0
	Neutral	Equilibrium mixture of amino and imino (1:9)	0.44	0.12
		Amino	8.7[d]	
		Imino	0.12	
	Anion	Mixture of $IIIb^-$ and VIa^- anions (6:4)	31.0	2.5
	$IIIb^-$		70.0	—
	$IIIa^-$		2.5	—
N^4-methoxycytidine (IIIb)	Cation		17.0	13.0
	Neutral	Mixture of amino and imino (1:20)	1.7	0.62
		Amino	53.0[d]	—
		Imino	0.62	—
	Anion		14.0	—
N^4-methyl-N^4-hydroxycytidine (VIa)	Cation		10.0	9.6
	Neutral	Mixture of amino tautomer and bipolar ion (3:7)	1.2	0.39
		Amino	5.5[d]	—
		Bipolar ion	0.39	—
	Anion		0.59	—
N^4-methyl-N^4-methoxycytidine (VIb)	Cation		17.5	15.0
	Neutral	Amino	38.0	35.0
$N_{(3)}$-methyl-N^4-hydroxycytidine (IXa)	Cation		2.9	2.6
	Neutral	Imino	1.5	1.3
$N_{(3)}$-methyl-N^4-methoxycytidine (IXb)	Cation		13.0	16.3
	Neutral	Imino	6.7	5.5

Irradiation intensities: 3×10^{15} quanta cm^{-2} s^{-1} at 254 nm; 4×10^{15} quanta cm^{-2} s^{-1} at 300 and 313 nm.

[a] The ratios of different tautomeric forms were taken from ref. 21.

[b] The relative error in the determination of ϕ did not exceed ±15%.

[c] Obtained at 300 nm.

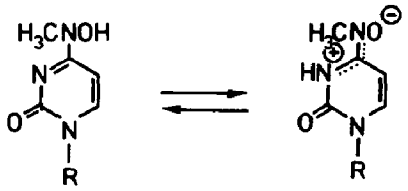
[d] Determined by calculation (see text).

tautomers than for imino tautomers in agreement with the data obtained for the fixed tautomers VIa, VIb, IXa and IXb.

A comparison of the photoreaction quantum yields for neutral amino and imino tautomers of N^6-methoxyadenosine, N^4-hydroxycytidine and

N^4-methoxycytidine indicates that the change from the purine to the pyrimidine nucleus has little effect on the efficiency of the photoreaction.

N^4-methyl-N^4-hydroxycytidine (VIa) exists in solution as an equilibrium mixture of the neutral amino tautomer and the bipolar ion in the ratio 3:7 [21]:



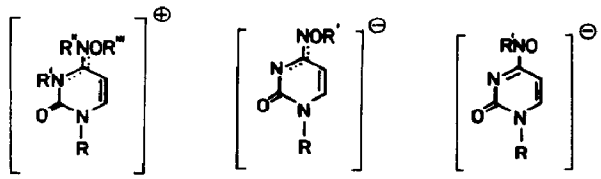
The quantum yield of photoreaction of the amino tautomer is significantly higher than that of the bipolar ion.

In general the introduction of a methyl group into a neutral molecule enhances the quantum yield of N—O bond rupture. For example, the transfer from hydroxy to methoxy derivatives is accompanied by an increase of fivefold to sixfold in the quantum yield for both the amino and the imino tautomers. The quantum yield is also enhanced by an order of magnitude when the methyl group is introduced at $N_{(3)}$. A decrease in the quantum yield value is observed only for the introduction of the methyl group at N^4.

Each cation of N^4-hydroxycytidine and its methyl derivatives exists in a single form which is similar for all the compounds investigated [21] (Fig. 5(a)). Therefore the quantum yields of N—O bond scission for these cations have similar values. The lowest value of the quantum yield for IXa^+ appears to be due to the combined effect of the presence of a methyl group at $N_{(3)}$ and the absence of such a group at the exocyclic hydroxy group. Protonation decreases the quantum yields for N^4-methoxycytidine amino tautomers (IIIb and VIb) but increases the yields for other compounds.

The quantum yield of N—O bond scission for anion $IIIb^-$ is significantly higher than that for VIa^-. Anion $IIIa^-$ exists in the solution in two forms (in the ratio 6:4) [21] similar to those of $IIIb^-$ and VIa^- (Figs. 5(b) and 5(c)) with quantum yields close to those for $IIIb^-$ and VIa^-.

In the case of compounds IXa and IXb it is necessary to consider the simultaneous existence in the solution of *syn* and *anti* isomers with different electronic structures [21] as well as the presence of different ionic states. When the quantum yields of the photoinduced isomerization and the rates of the spontaneous *syn* $\rightleftarrows$ *anti* transitions via the cation [21] as well as the intensity of the irradiating light (about 2 quanta s^{-1} per nucleoside) are taken into account it can be reduced that the photoinduced scission of the N—O bond at pH < 4 is characteristic of the "dark" equilibrium (about 95% *syn* isomer [21]) whereas that at pH > 9 is characteristic of the "light" equilibrium (about 60% - 70% *anti* isomer depending on the irradiating wavelength [21]).



(a) (b) (c)

Fig. 5. (a) Cations of N^4-hydroxycytidine and its methyl derivatives (IIIa$^+$, R′ ≡ R″ ≡ R‴ ≡ H; IIIb$^+$, R′ ≡ R″ ≡ H, R‴ ≡ CH_3; VIa$^+$, R′ ≡ R‴ ≡ H, R″ ≡ CH_3; VIb$^+$, R′ ≡ H, R″ ≡ R‴ ≡ CH_3; IXa$^+$, R′ ≡ CH_3, R″ ≡ R‴ ≡ H; IXb$^+$, R′ ≡ R‴ ≡ CH_3, R′ ≡ H); (b), (c) anions of N^4-hydroxycytidine and its methyl derivatives ((b) IIIa$^-$, R′ ≡ H; IIIb$^-$, R′ ≡ CH_3; (c) IIIa$^-$, R′ ≡ H; VIa$^-$, R ≡ CH_3).

However, the rate of N—O bond rupture is unaffected by changing the wavelength of the irradiating light from 254 to 313 nm or by changing the pH from 3.5 to 13, despite the significant difference in the UV spectra of the *syn* and *anti* isomers in the interval 254 - 313 nm. Therefore the difference in the electronic structures of the *syn* and *anti* isomers has no effect on the efficiency of N—O bond scission.

4. Conclusion

A number of compounds containing N—O bonds are known which undergo both photoinduced rearrangements of different types and photoreduction (Fris [32] and Beckmann [33, 34] photorearrangements, phototransformations of *N*-oxides [11, 35, 36], photoreduction of oximes [37], Barton reactions [38, 39] etc.). The intermediate step in the rearrangement of *N*-oxides, nitrones and oximes appears to be the formation of oxaziridines [11, 35, 40]. However, the photoreduction of *N*-oxides probably proceeds through the heterolytic dissociation of the N—O bond as an intermediate step, while the photoreaction of oximes and nitrosoesters proceeds through the homolytic dissociation of the N—O bond. The photoinduced transformation of the N^4- and N^6-hydroxy derivatives, and their methyl-substituted derivatives, of cytidine and adenosine studied in this work can be considered as photoreduction proceeding via the homolytic dissociation of the N—O bond. However, owing to the type of N—O bond, these compounds differ significantly from both *N*-oxides and nitrosoesters, and the most active amino form of the *N*-hydroxy derivatives of cytidine and adenosine is closer to the hydroxamic acids or the *N*-arylhydroxylamines than to the oximes.

We now consider the correlation of the photoreactions investigated with calculations of the electronic structure of the compounds [21]. Only those data which are relevant to the photoreaction of interest are analysed.

As the reaction proceeds in the T_1 state, the localization of excitation and the potential photoreactivity of the molecule can be characterized by the distribution of the spin density ρ on the atoms (the localization of excitation, *i.e.* the change in the electronic structure on excitation, correlates

with the localization of the ρ values). The values of ρ for the nitrogen and oxygen atoms of the N—O bond of the cytosine and adenine analogues investigated here are given in Table 6.

Table 6 shows that in most of the ions and tautomers investigated in this work a large part of the spin density (*i.e.* the excitation) is strongly localized on the N—O fragment and exceeds the mean value of $2/m$ for atoms of this group (equal to 0.22 for cytosine derivatives and 0.18 for adenine derivatives) where m is the number of atoms in the π electron system of the molecule. Thus the theory correctly predicts the possibility of rupture of the N—O bond for some of the compounds. However, there are substantial discrepancies between theoretical predictions and experimental data. Thus, according to the calculations localization of the spin density on nitrogen and oxygen atoms in neutral molecules occurs only for imino forms and the ρ values for amino forms are low. Nevertheless, the rupture of the N—O bond is shown experimentally to be more efficient for amino forms than for imino forms. Such disagreement between theory and experiment appears to be related to inadequacies in the semiempirical method of calculation, *i.e.* the approximation used (complete neglect of differential overlap (CNDO/S)) or its parametrization does not satisfactorily describe the real situation. In our opinion this discrepancy should stimulate the development of an improved approach (the application of better methods of calculation, the inclusion of factors associated with the photoreaction mechanism etc.).

The reactivity of a compound is determined not only by its electronic structure but also by a number of other factors. One of the factors determining the efficiency of photoreactions is the population density of the corresponding excited state. UV irradiation of uridine and cytidine in dilute aqueous solutions results in the photohydration of the $C_{(5)}$—$C_{(6)}$ bond which occurs in the S_1 state [22]. According to calculations [21, 30], the transition of amino and imino tautomers of N^4-hydroxycytosine into the S_1 state is accompanied by almost the same change in the electronic structure of the molecular fragment $C_{(5)}$—$C_{(6)}$ as that found for cytosine and uracil. However, the photohydration of N^4-hydroxycytidine and its derivatives did not occur under the conditions required for rupture of the N—O bond [41]. As the rupture of the N—O bond occurs in the T_1 state, the lack of concomitant photohydration of these substances is probably due to a significant increase in the $S_1 \rightarrow T_1$ transition, *i.e.* by a decrease in the population density of the S_1 state accompanied by an increase in the population density of the T_1 state. This phenomenon may also provide an explanation for the higher quantum yield of N—O bond rupture for the amino tautomer in comparison with that for the imino tautomer. In fact, according to calculations [30] the energy difference between the S_1 and T_1 states for the amino tautomer is much lower than that for the imino tautomer. Furthermore, the T_1 energy of the amino tautomer of N^4-hydroxycytosine is much higher than that of the imino tautomer. Both these facts may favour the higher population density of the T_1 state of the amino tautomer compared with that of the imino tautomer.

TABLE 6

Distribution of the spin density ρ on atoms of the exocyclic *N*-hydroxy group of N^4-hydroxycytosine and N^6-hydroxyadenine in the T_1 state

Compound	*Ionic form*	*Tautomeric form*	*Atom*	
			N	*O*
N^4-hydroxycytosine	Cation		0.23	0.04
	Neutral	Amino	0.04	0.00
		Imino	0.41	0.06
		Bipolar ion	0.41	0.48
	Anion	IIIb$^-$	0.55	0.07
		VIa$^-$	0.42	0.31
N^6-hydroxyadenine	Cation	Tautomer protonated on pyridine ring	0.32	0.04
		Imino tautomer protonated at imidazole ring	0.54	0.11
		Amino tautomer protonated at imidazole ring	0.32	0.05
	Neutral	Imino	0.18	0.06
		Amino	0.19	0.00
	Anion		0.54	0.07

The other factor affecting the quantum yield of the photoreaction is the reversibility of the intermediate stages. In the bipolar ion and in anions IIIb$^-$ and VIa$^-$ the spin density is strongly localized on the exocyclic nitrogen and oxygen atoms (Table 6). However, the quantum yield of N—O bond rupture for the type IIIb$^-$ anion is significantly higher than those for the bipolar ion and the type VIa$^-$ anion (Table 5). This appears to be due to the formation of a very active oxygen anion radical as an intermediate stage in the case of the last two ions. This intermediate radical encourages an effective recombination to form the starting compound, *i.e.* the effective quantum yield is decreased.

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