Journal of Photochemistry, *20* (1982) 229 - 252

PHOTOCHEMISTRY OF N^6-METHOXYADENOSINE AND OF N^4-HYDROXYCYTIDINE AND ITS METHYL DERIVATIVES I: SPECTROSCOPIC AND QUANTUM CHEMICAL INVESTIGATION OF IONIC AND TAUTOMERIC FORMS: *SYN–ANTI* ISOMERIZATION

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(Received March 10, 1982)

Summary

The individual absorption spectra of various ionic and tautomeric species and the corresponding equilibrium constants for N^6-methoxyadenosine and for N^4-hydroxycytidine and its methyl derivatives were determined by computer analysis of the integrated spectra of these compounds measured at different pH values. The individual spectra of the ionic, tautomeric and isomeric species were decomposed into bands corresponding to the separate electronic transitions.

Quantum chemical calculations of the electronic structure and spectra of all the ionic, tautomeric and isomeric species of the compounds studied were carried out using the complete neglect of differential overlap (CNDO/S) and Pariser–Parr–Pople approximations. The calculated results are in good agreement with the experimental spectral and photochemical data.

The photoinitiated and dark *syn–anti* isomerization reactions of $N_{(3)}$-methyl-N^4-hydroxycytidine and $N_{(3)}$-methyl-N^4-methoxycytidine were investigated theoretically and experimentally.

1. Introduction

The substitution of a hydrogen atom in the exocyclic amino group of adenosine and cytidine by a hydroxy or a methoxy group leads to significant alterations in the chemical, photochemical and functional properties of these compounds. Thus the UV irradiation of neutral aqueous solutions of N^6-methoxyadenosine, N^4-hydroxycytidine and N^4-methoxycytidine produces an unusual reaction: the N—O bond ruptures and cytidine and adenosine are formed [1]. The *N*-hydroxy derivatives of cytidine and adenosine have an

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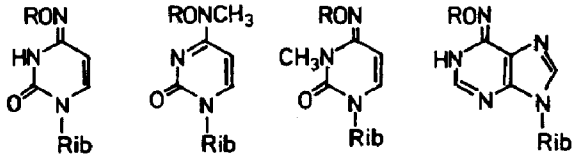
ambiguous specificity in the template systems of polynucleotide synthesis and their formation is the main reason for the mutagenic action of hydroxylamine [2].

A knowledge of the electronic structure, the spectroscopic properties and the ionic, tautomeric and *syn–anti* isomeric equilibria is necessary to understand the unusual photochemical [1] and functional [2] behaviour of the compounds investigated. Therefore we have carried out a detailed experimental and quantum chemical study of the electronic structure and the spectroscopic and photochemical properties of various ionic, tautomeric and stereoisomeric species of N^6-hydroxyadenosine and of N^4-hydroxycytidine and its methyl derivatives.

2. Materials and methods

Adenosine and cytidine (Calbiochem, grade A) and the other commercial reagents (chemically pure grade and special chemically pure grade) were used without additional purification. $N_{(3)}$-methylcytidine was prepared as described in ref. 3, and N^4-hydroxycytidine (Ia), N^4-methoxycytidine (Ib) and N^6-methoxyadenosine (IV) were prepared as described in ref. 1. The preparation and purification of N^4-methylcytidine, N^4-methyl-N^4-hydroxycytidine (IIa), N^4-methyl-N^4-methoxycytidine (IIb), $N_{(3)}$-methyl-N^4-hydroxycytidine (IIIa) and $N_{(3)}$-methyl-N^4-methoxycytidine (IIIb) are described in ref. 4. The structures of the parent compounds I - IV are shown in the following scheme:



I II III IV

(Rib ≡ β-D-ribofuranosyl; **a**, R ≡ H; **b**, R ≡ CH_3).

UV absorption spectra were recorded at pH values of 1 - 14 and a temperature of 25 ± 1 °C using a Hitachi EPS-3T double-beam spectrophotometer. Circular dichroism (CD) spectra were obtained using a Jobin–Yvon model 3 dichrograph and D_2O proton magnetic resonance (PMR) spectra were obtained using a Varian XL-100 spectrometer. The nuclear Overhauser effect (NOE) was used to determine the orientation of the methoxy group relative to the $H_{(5)}$ proton in isomer IIIb [5]. The effects of the methoxy group protons were suppressed during the NOE measurements for the $H_{(5)}$ protons.

The molar extinction coefficients ϵ for the UV absorption of N^4-methylcytidine and $N_{(3)}$-methylcytidine were obtained from refs. 6 and 7 respectively. The ϵ values of the other compounds were determined at the isosbestic points of the absorption spectra of reaction mixtures for the phototrans-

formation of the parent compounds I, II, III and IV into cytidine, N^4-methylcytidine, $N_{(3)}$-methylcytidine and adenosine respectively [1, 4].

The mathematical methods used to analyse the spectral data (integrated absorption spectra at various pH values) in order to obtain the individual absorption spectra and equilibrium constants of the ionic and tautomeric species and the methods of decomposing individual spectra into bands corresponding to separate electronic transitions have been described elsewhere [8, 9].

The absorption spectra of the *syn* and *anti* isomers of N^4-hydroxy-$N_{(3)}$-methylcytidine and N^4-methoxy-$N_{(3)}$-methylcytidine which coexist in solution were determined as follows. The problem was initially solved for a mixture of two components A and B with extinction coefficients ϵ_A and ϵ_B respectively. The relative concentrations of A and B in the solution are denoted by α and $1 - \alpha$ respectively. Then the extinction coefficients ϵ_k^{λ} in the integrated spectra for two wavelengths where k is the irradiation time governing ϵ^{λ} can be written as

$$\epsilon_1^{\lambda_1} = (1 - \alpha_1)\epsilon_B^{\lambda_1} + \alpha_1\epsilon_A^{\lambda_1}$$
$$\epsilon_1^{\lambda_2} = (1 - \alpha_1)\epsilon_B^{\lambda_2} + \alpha_1\epsilon_A^{\lambda_2} \quad (1)$$

which gives

$$\frac{\epsilon_1^{\lambda_1} - \epsilon_B^{\lambda_1}}{\epsilon_1^{\lambda_2} - \epsilon_B^{\lambda_2}} = \frac{\epsilon_A^{\lambda_1} - \epsilon_B^{\lambda_1}}{\epsilon_A^{\lambda_2} - \epsilon_B^{\lambda_2}} \quad (2)$$

If $\epsilon_B^{\lambda_1} = 0$ when $\lambda = \lambda_1$, then

$$\epsilon_A^{\lambda_2} - \epsilon_B^{\lambda_2} = \epsilon_A^{\lambda_1}\frac{\epsilon_1^{\lambda_2} - \epsilon_B^{\lambda_2}}{\epsilon_1^{\lambda_1}} \quad (3)$$

and simiiarly

$$\epsilon_A^{\lambda_2} - \epsilon_B^{\lambda_2} = \epsilon_A^{\lambda_1}\frac{\epsilon_2^{\lambda_2} - \epsilon_B^{\lambda_2}}{\epsilon_2^{\lambda_1}} \quad (4)$$

for an integrated spectrum for other concentrations of species A and B. Then from eqns. (3) and (4) we obtain

$$\epsilon_B^{\lambda_2} = \frac{\epsilon_1^{\lambda_2}\epsilon_2^{\lambda_1} - \epsilon_1^{\lambda_1}\epsilon_2^{\lambda_2}}{\epsilon_2^{\lambda_1} - \epsilon_1^{\lambda_1}} \quad (5)$$

We obtained the absorption spectra $f_B(\lambda)$ of component B by determining ϵ_B^{λ} at various values of λ. In order to use this procedure it must be confirmed that $\epsilon_B = 0$ at $\lambda = \lambda_1$. Methods of determining the value of λ_1 that satisfies this condition have been described in detail elsewhere [10].

This method can only be used to determine $f_B(\lambda)$ when it is fully overlapped by the spectrum $f_A(\lambda)$ of the other component, *i.e.* for $f_B(\lambda)$ such that there is no value of λ for which $\epsilon_A^{\lambda} = 0$. Thus $f_A(\lambda)$ and the percentage content of the components of the mixture cannot be rigorously determined. However, these values can be approximated as follows. When the absorption spectrum $f_k(\lambda)$ of a mixture is decomposed into bands corresponding to

separate electronic transitions [9], all the parameters of the absorption bands of component B are fixed except for the intensity which is proportional to the percentage content of B in the solution. The only absorption bands determined for component A are those with parameters (halfwidth and asymmetry) which are specific for the class of compounds studied and with spectral positions which agree with quantum chemical calculations. The percentages of components A and B in the different mixtures are determined by the least-squares method using $f_A(\lambda)$ and $f_B(\lambda)$ as reference spectra and the integrated spectra are calculated from

$$f_k(\lambda) = \alpha_k f_A(\lambda) + (1 - \alpha_k) f_B(\lambda) \tag{6}$$

These spectra are compared with the experimental spectra. If they disagree by more than the experimental error $f_A(\lambda)$ is adjusted and the procedure is repeated until the deviation becomes less than the experimental error. Further refinement is unnecessary.

α_k and $1 - \alpha_k$ can also be determined from independent PMR measurements. The values found by this method correlate well with those obtained by the method discussed in the preceding paragraph.

The UV irradiation dose used in the investigation of the photoinduced *syn–anti* isomerization of $N_{(3)}$-methyl-N^4-hydroxycytidine and $N_{(3)}$-methyl-N^4-methoxycytidine was such that less than 1% of the starting compound underwent other phototransformations [4]. Under these conditions the solution can be assumed to contain only *syn* and *anti* isomers and the following equation can be written:

$$\frac{ds}{dt} = -\phi_{sa} I_0\{1 - \exp(-\epsilon_s s l)\} + \phi_{as} I_0\{1 - \exp(-\epsilon_a a l)\} + K_{as} a - K_{sa} s \tag{7}$$

where s, ϵ_s, a and ϵ_a are the concentrations and molar extinctions at the wavelength of irradiation of the *syn* and *anti* isomers, ϕ_{sa} and ϕ_{as} are the quantum yields of the photoinduced *syn–anti* and *anti–syn* isomerizations, K_{sa} and K_{as} are the rate constants of the dark *syn–anti* and *anti–syn* isomerizations, I_0 is the intensity of the incident light and l is the optical path length.

If the rate of dark isomerization is negligible in comparison with the rate of photoinduced isomerization, the last two terms on the right-hand side of eqn. (7) can be disregarded. Then after attainment of "light" dynamic equilibrium ($ds/dt = 0$) we have

$$\frac{\phi_{as}}{\phi_{sa}} = \frac{1 - \exp(-\epsilon_s s_p l)}{1 - \exp(-\epsilon_a a_p l)} \tag{8}$$

where s_p and a_p are the equilibrium concentrations of the *syn* and *anti* isomers.

The integration of eqn. (7) is quite complex even if the dark isomerization is negligible. However, if a is less than 0.15 the second term on the right-hand side of this equation is much less than the first term and can be neglected. Integration of eqn. (7) with these approximations gives

$$\phi_{sa} = \frac{1}{\epsilon_s l I_0 t} \ln\left\{\frac{\exp(D_s^0) - 1}{\exp(D_s^t) - 1}\right\} \tag{9}$$

where t is the time of irradiation and D_s^0 and D_s^t are the optical densities of the *syn* isomer before and after irradiation for t min.

ϕ_{as} is obtained by substituting eqn. (9) into eqn. (8). The intensity of the incident light flux was determined by uridine actinometry at 254 nm and by ferrioxalate actinometry at 313 nm. The quantum yield of uridine photohydration is equal to 2.16×10^{-2} [11].

The rate constants of the dark *syn–anti* and *anti–syn* isomerizations are determined by solving

$$\frac{ds}{dt} = K_{as}a - K_{sa}s$$
$$s + a = C_0 \tag{10}$$

for the conditions

$$K_{sa} = K_{as}\frac{a_\infty}{s_\infty} \qquad t \to \infty$$
$$a = a_0 \qquad s = s_0 \qquad t \to 0 \tag{11}$$

After integration of eqn. (10) under these conditions we obtain

$$K_{as} = \frac{s_\infty}{tC_0} \ln\left(\frac{s_0 - s_\infty}{s - s_\infty}\right) \tag{12}$$

The quantum chemical calculations of the electronic structure and spectra of the ionic and tautomeric species and the *syn* and *anti* isomers were carried out using the complete neglect of differential overlap (CNDO/S) method [12] and the Pariser–Parr–Pople (PPP) π electron approximations. The parameters used for the PPP calculation were obtained from refs. 13 and 14 and were those valid for the resonance molecular structures closest to those obtained by the CNDO/S method.

The geometries of the amino and imino forms were assumed to be identical with those of cytosine and adenine and those of uracil and hypoxanthine respectively. The correctness of this assignment of the unknown geometry is supported by the fact that the geometries of 1,5-dimethyl-N^4-hydroxycytosine [15] and $N_{(1)}$-methyl-N^4-hydroxycytosine [16] determined from X-ray data are quite similar to those used in this work. The geometry of the exocyclic substituents was taken from refs. 15 - 20. The following bond lengths were used: N—H, 1.04 Å; O—H, 0.96 Å; C—H, 1.08 Å; C—H in the methyl group, 1.09 Å [21]. The length of the bonds between the nitrogen atoms and the carbon atoms of the methyl groups attached to them was taken as 1.47 Å. It was also assumed that the angles of the bonds between the hydrogen atoms and the nitrogen and carbon atoms in the heterocycles are equal, and that the angle between the N—O and O—H bonds in exocyclic substituents is 105°.

The influence of the methyl group attached to the nitrogen and oxygen atoms was generally ignored in the quantum chemical calculations since such a substitution stabilizes certain tautomeric forms but has only a slight effect on the electronic structure and the molecular spectra. The methyl group was taken into account only in those cases when it interacts directly with other parts of the molecule (*e.g.* for N^4-hydroxy-$N_{(3)}$-methylcytidine and N^4-methoxy-$N_{(3)}$-methylcytidine where the interaction of the $N_{(3)}$-methyl group with the oxygen atom of the exocyclic hydroxy or methoxy group is possible). In this case one of the hydrogen atoms of the methyl group is positioned in the plane of the molecule and the two others are located above and below it.

3. Results and discussion

3.1. Acid–base equilibria

The main ionic and tautomeric forms of the compounds studied are presented in Figs. 1 and 2. The enol forms derived from the O^2 of the pyrimidine ring are omitted as they are unlikely to exist [9]. Anion (5) was not found experimentally and structure (4) was detected for compound **IIa** only.

Table 1 shows that the pK values obtained by computer analysis of the spectral data are in good agreement with the published data. The decrease in pK_1 when a hydroxy or methoxy group is attached to the exocyclic nitrogen atom of the cytosine ring does not exceed unity in the amino forms (compare **IIa** and **IIb** with cytidine and N^4-methylcytidine) but is greater than 7 units in the imino forms (compare **IIa** and **IIIb** with $N_{(3)}$-methylcytidine). Such a large difference in the effect of these substituents on the pK_1 value

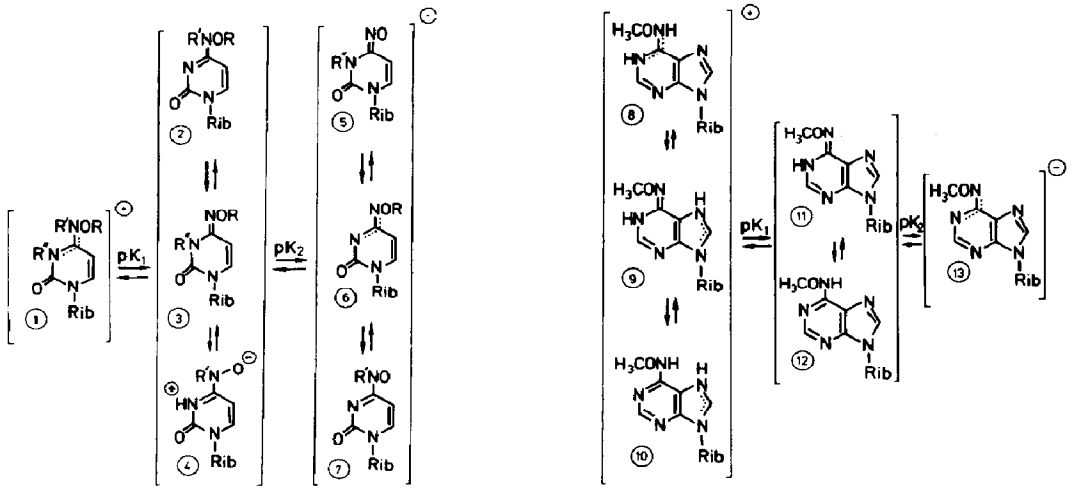


Fig. 1. Ionic and tautomeric equilibria for N^4-hydroxycytidine and its methyl derivatives (Rib ≡ β-D-ribofuranosyl; R, R′, R″ ≡ H or CH_3).

Fig. 2. Ionic and tautomeric equilibria for N^6-methoxyadenosine (Rib ≡ β-D-ribofuranosyl).

TABLE 1

pK values of the compounds investigated

Nucleoside	pK_1	pK_2	*Published value*
N^4-methylcytidine	3.99	—	3.92 [22]
$N_{(3)}$-methylcytidine	8.41	—	8.70 [3]
N^4-hydroxycytidine (Ia)[a]	2.29	10.22	2.26 [23], 2.3 [24] 10.5 [23]
N^4-methoxycytidine (Ib)[a]	0.98	11.04	1.18 [25]
N^4-methyl-N^4-hydroxycytidine (IIa)[a]	3.55	8.61	—
N^4-methyl-N^4-methoxycytidine (IIb)[a]	3.05	—	3.05 [25]
$N_{(3)}$-methyl-N^4-hydroxycytidine (IIIa)[a]	1.06	—	—
$N_{(3)}$-methyl-N^4-methoxycytidine (IIIb)	1.20	—	1.53 [25]
N^6-methoxyadenosine (IV)[a]	2.95	10.24	—

[a]Tautomeric and *syn–anti* transformations are possible for these compounds; therefore their effective pK values are given.

is due to their being bound directly to the centre of protonation in the imino forms. The comparatively small difference in the pK_1 values for adenosine (pK_1 = 4.19 [9]) and N^6-methoxyadenosine may be due to the presence of a high percentage of amino form (see below) and/or to the protonation of IV at the imidazole ring.

The pK_2 value of **Ia** is lower than that of **Ib**. As structure (4) was not observed for **Ia**, this difference is due to deprotonation of N^4—H in the amino form (structure (2)) and/or of $N_{(3)}$—H in the imino form (structure (3)). Deprotonation of the NOH group, which appears to proceed only for the amino forms, is also possible in **Ia**. This is confirmed by the fact that deprotonation was not observed up to pH 14 for the imino form of **IIIa** (structure (3)), but the amino form, which is an analogue of hydroxamic acid, is deprotonated in a weak alkaline medium. Moreover, the amino form of **IIa** predominantly exists in aqueous solution as a bipolar ion, *i.e.* the $N_{(3)}$ atom of the pyrimidine ring which has a low basicity successfully competes for the proton with the exocyclic N—O group of the amino form.

There appear to be three reasons for the difference in the pK_2 values observed for **IIa** and **Ia**.

(1) There is more of the easily deprotonating amino form in neutral aqueous solutions of **IIa** than in similar solutions of **Ia**.

(2) The insertion of the methyl group at the exocyclic nitrogen atom decreases the pK_2 value of the hydroxyamino group.

(3) The electronic structure of the pyrimidine ring of the bipolar ion is intermediate between those of the neutral uridine molecule and the cytidine cation [9, 26]. Therefore the deprotonation of structure (4) at $N_{(3)}$ will proceed significantly more easily than that of the imino form of N^4-hydroxycytidine (structure (3)).

The marked decrease in the pK_2 value of N^6-methoxyadenosine (IV) compared with that of adenosine arises on the one hand from the effect of the methoxy group on the deprotonation at N^6 of structure (12) and on the other hand from the possible absence of the distinct difference between the pK value for deprotonation at $N_{(1)}$ in structure (11) and the corresponding pK for inosine (pK_2 = 8.86 [9]). The electronic structure of the neutral inosine molecule is similar to that of (11) and the anion structure is similar to structure (13).

3.2. UV spectroscopy and tautomeric equilibria

The absorption spectra for various ionic forms of the compounds studied and the decomposition of these spectra into bands corresponding to separate electronic transitions are shown in Figs. 3 - 7. The initial absorption spectra and the theoretical integrated spectra are presented in the same

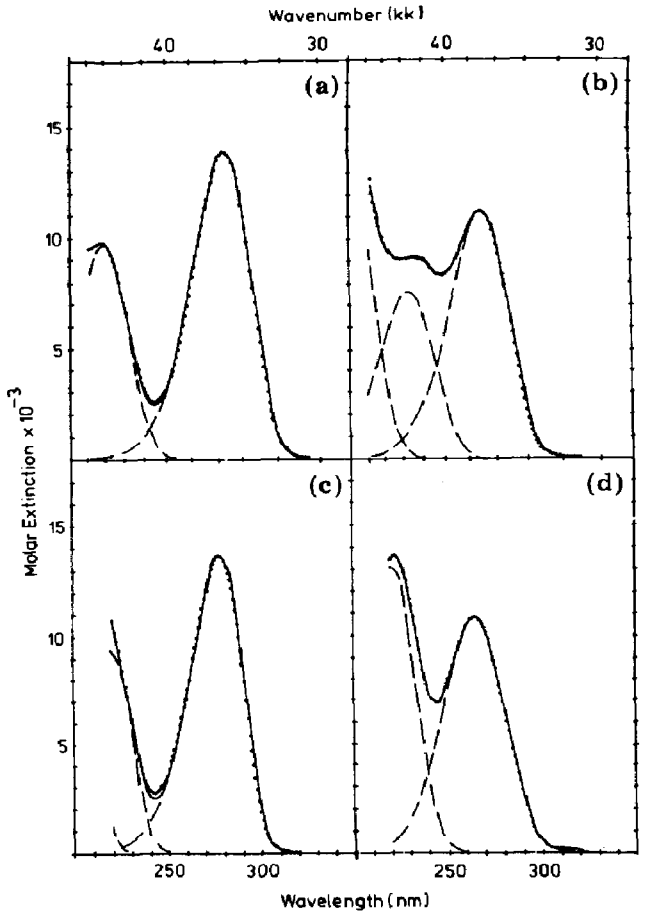


Fig. 3. UV absorption spectra of (a) N^4-methylcytidine cations, (b) N^4-methylcytidine molecules, (c) methylcytidine cations and (d) methylcytidine molecules: ●, experimental data; — — —, separate electronic transitions; ——, superposed bands (envelope curve).

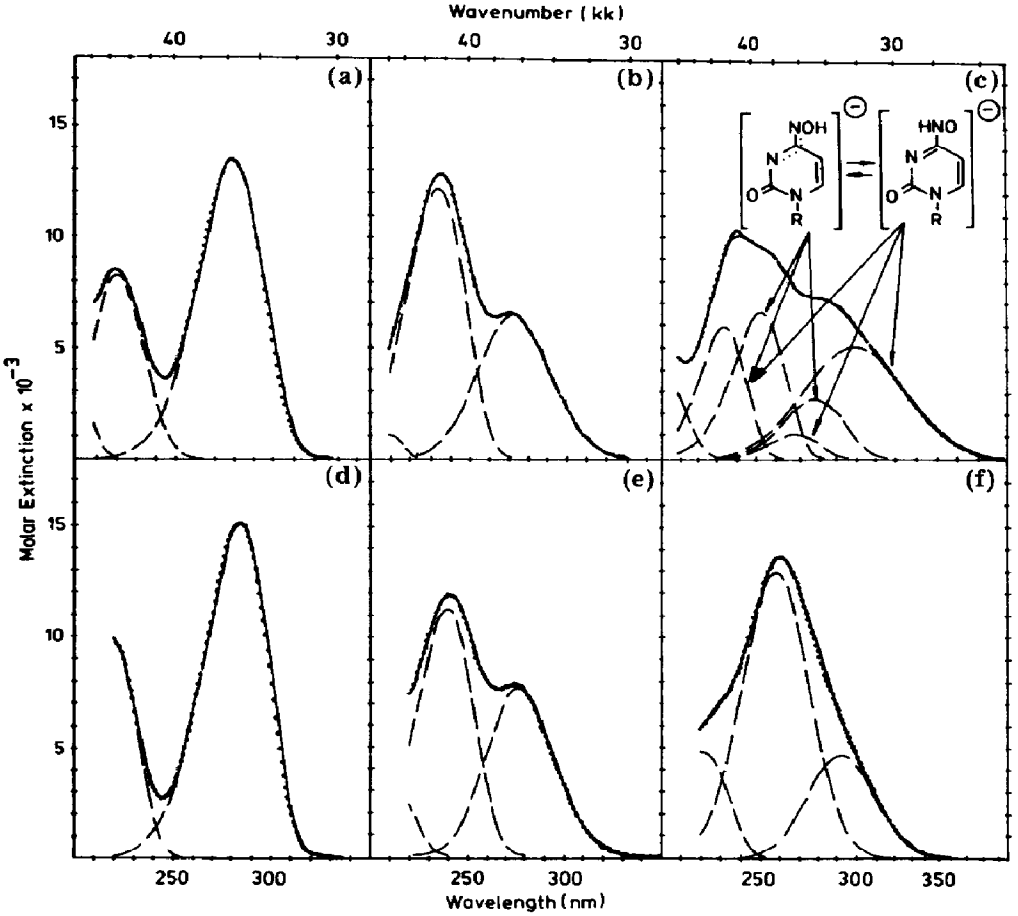


Fig. 4. UV absorption spectra of (a) N^4-hydroxycytidine cations, (b) N^4-hydroxycytidine molecules (predominantly imino tautomer), (c) an equilibrium mixture (6:4) of anionic tautomers (6) and (7) of N^4-hydroxycytidine, (d) N^4-methoxycytidine cations, (e) N^4-methoxycytidine molecules (predominantly imino tautomer) and (f) anionic tautomer (6) of N^4-methoxycytidine. The symbols are as defined in Fig. 3.

figure both for clarity and to demonstrate any discrepancies between experimental and theoretical data.

Tables 2 and 3 give the theoretical (quantum chemical calculations) and experimental (computer analysis of the spectral data) values of the electronic transition energies E for various ionic and tautomeric species of the compounds studied, the oscillator strengths f for the corresponding π electron transitions and the radiation lifetimes τ_0 for the lowest excited singlet states of the molecules. The quantum chemical calculations were performed for bases and the experimental data were obtained for corresponding nucleosides with non-ionized carbohydrate residues. It is known that the transition from base to nucleoside is accompanied by some changes in the band positions of the absorption spectra; however, these shifts do not generally exceed 0.1 eV [9].

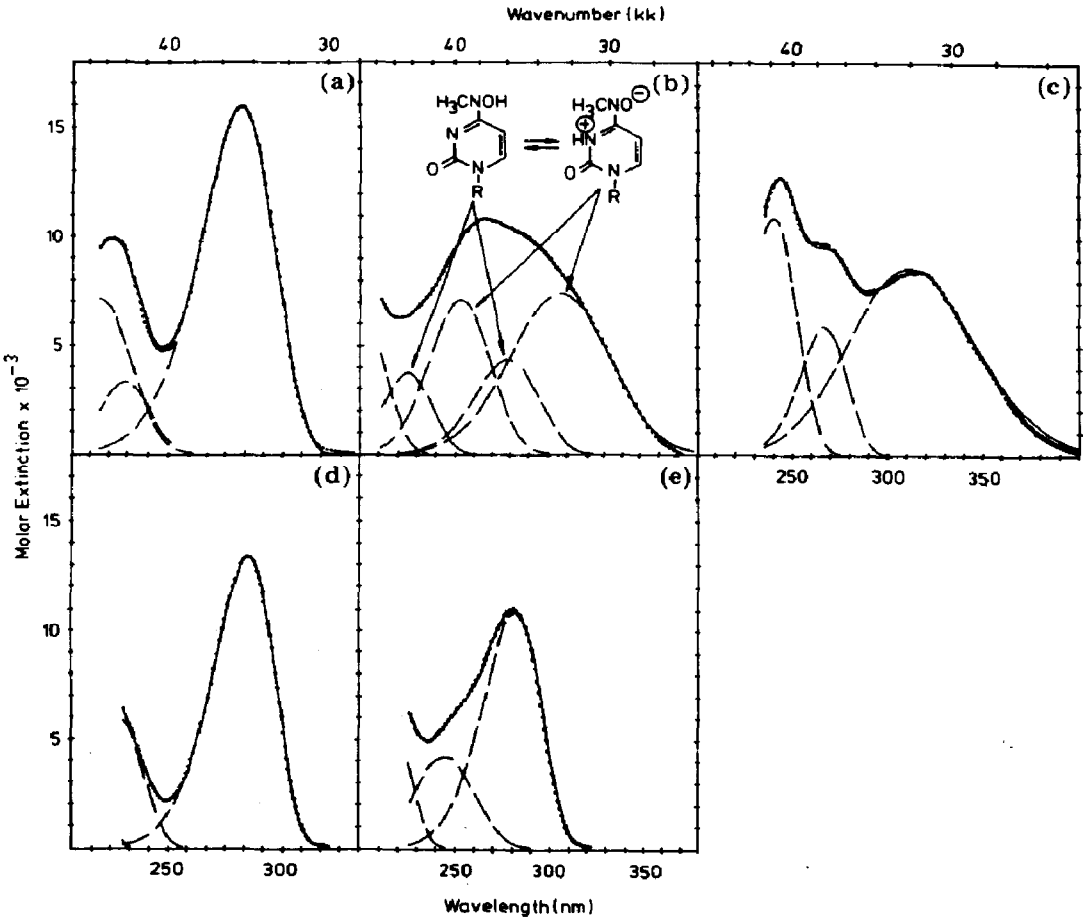


Fig. 5. UV absorption spectra of (a) N^4-methyl-N^4-hydroxycytidine cations, (b) an equilibrium mixture (3:7) of neutral amino tautomer (2) and bipolar ion (4) of N^4-methyl-N^4-hydroxycytidine, (c) N^4-methyl-N^4-hydroxycytidine molecules, (d) N^4-methyl-N^4-methoxycytidine molecules and (e) neutral amino tautomer (2) of N^4-methyl-N^4-methoxycytidine. The symbols are as defined in Fig. 3.

The calculated values of f are not given in these tables because the quantum chemical approximations used produce data with large errors [9]. In some cases the E values determined were insufficiently reliable for various reasons (low intensity of the absorption band, strong overlapping of the adjacent bands, shortage of experimental information etc.).

The difference between the theoretical and experimental energies for the longest absorption bands does not usually exceed 0.3 eV. Within this accuracy there is also a reasonable correlation between the results of the π electron and the CNDO/S calculations. Both calculation methods correctly reproduce the large spectral shifts connected with the changes in the ionic or tautomeric state of the molecules. The only discrepancy exists for the PPP calculation for anion (6). This may be due to the fact that the calculations in this approximation are performed on the basis of a single resonance struc-

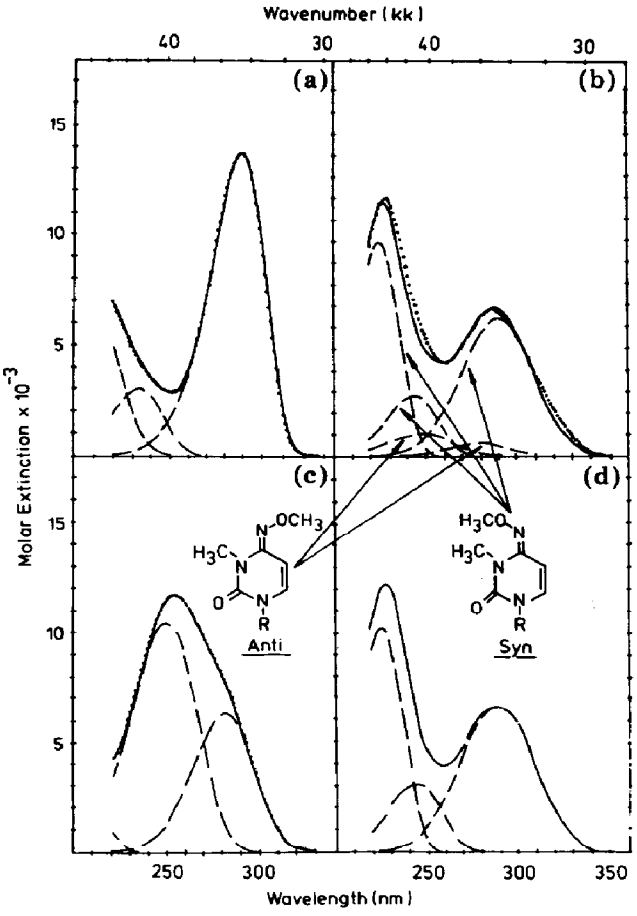


Fig. 6. UV absorption spectra of the ionic forms of $N_{(3)}$-methyl-N^4-methoxycytidine: (a) cations; (b) an equilibrium mixture of neutral *syn* and *anti* isomers (95% *syn*); (c) *anti* isomer; (d) *syn* isomer. The symbols are as defined in Fig. 3. Few experimental data are available for the *syn* isomer (see Section 2).

ture, whereas the electronic structure of this tautomer cannot be adequately described in terms of a single resonance structure.

The theoretical results for the second absorption bands are poorer than those for the first absorption band in both methods; however, the CNDO/S approximation produces results that are closer to the experimental values. The discrepancy between the theoretical results is particularly marked for cations IIIa, IIa, Ia and Ib. This discrepancy appears to be related to an incorrect representation of the σ core in the PPP approximation for cations in particular [9].

The attachment of the methyl group to the $N_{(3)}$ or the N^4 atom of cytidine leads to a very small shift in the spectral position of the first absorption band. The introduction of a hydroxyl at the N^4 atom of cytidine markedly enhances the influence of the methyl group on the absorption spectra. The substitution of a hydrogen atom at $N_{(3)}$ or N^4 or in the exocyclic

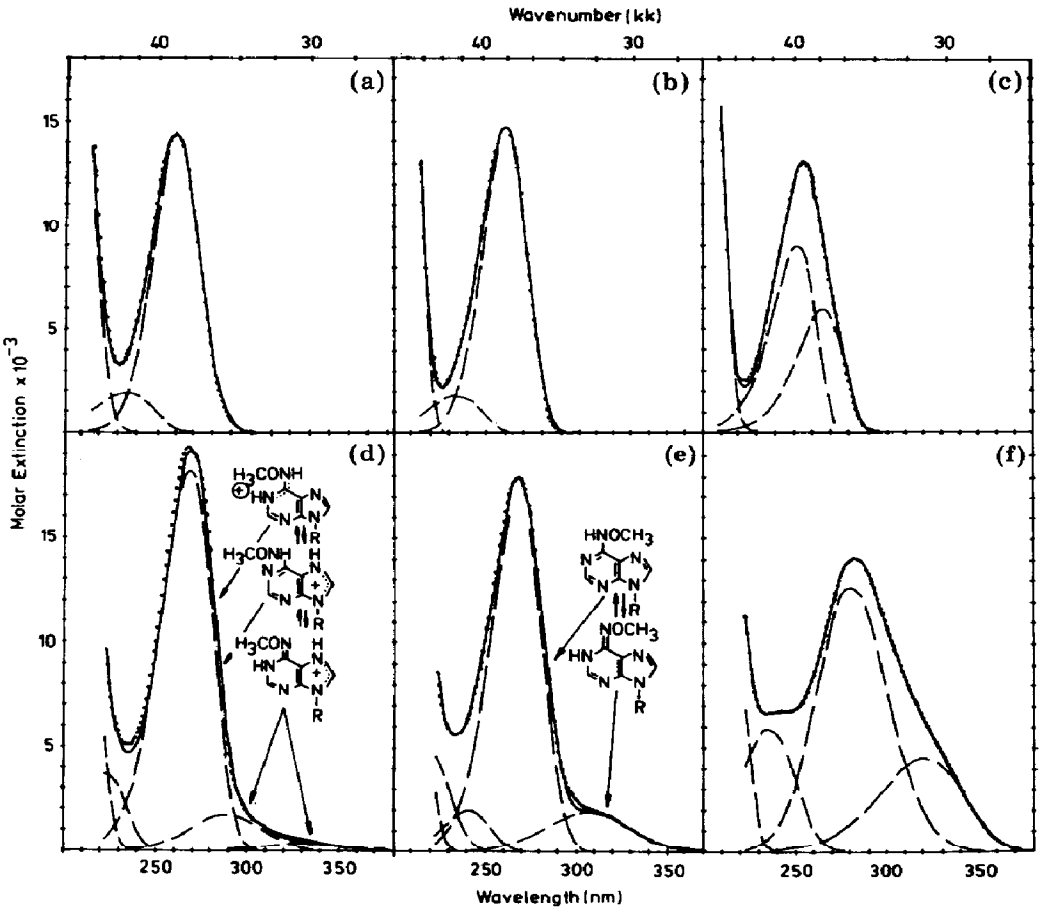


Fig. 7. UV absorption spectra of (a) adenosine cations, (b) adenosine molecules, (c) inosine anions, (d) an equilibrium mixture of *N*-methoxyadenosine cationic tautomers (8), (9) and (10), (e) an equilibrium mixture of amino (12) and imino (11) tautomers of *N*-methoxyadenosine in the ratio 7:3 and (f) *N*-methoxyadenosine anions. The symbols are as defined in Fig. 3.

hydroxyl group on the methyl group leads to average additive spectral shifts of −0.08 eV, −0.12 eV or −0.05 eV respectively.

The longest absorption band of the neutral molecules of $N_{(3)}$-methylcytidine and N^4-methylcytidine is hypsochromically shifted for the imino forms compared with that for the amino form with a spectral position similar to that of cytidine. The ratio f_1/f_2 of the oscillator strengths for the first two transitions in N^4-methylcytidine is greater than unity while that for $N_{(3)}$-methylcytidine is less than unity. A similar oscillator strength ratio was found for N^4-hydroxycytidine and its methyl derivatives. All amino forms, regardless of their ionic state, have f_1/f_2 greater than unity, as for cytidine [9]. However, for neutral imino forms and their *anti* isomers f_1 is less than f_2 as for uracil and its nucleosides and nucleotides [9]. It should be emphasized that f_1 is greater than f_2 for neutral *syn* isomers of **IIIa** and **IIIb** although

they are imino tautomers. This behaviour is shown in the spectroscopic appearance of the interaction of the hydroxy and methoxy groups with the methyl group at the $N_{(3)}$ atom in the case of *syn* isomers (see below).

Decomposition of the individual absorption spectra of the ionic forms into bands corresponding to separate electronic transitions enabled us to determine some of the ratios between the tautomers. Thus a comparison of the absorption spectra of anion **Ib** (fixed tautomer (6)) and anion **IIa** (fixed tautomer (7)) with the results of the decomposition of the integrated spectra of the mixture of tautomeric anions of **Ia** gives the ratio of imino to amino tautomers for this mixture as 6:4. Solutions of neutral molecules of **Ia** and **Ib** contain low concentrations of the amino form [27]. This fact, together with the closeness of the absorption bands of the amino and imino tautomers (according to quantum chemical calculations), made it impossible to isolate amino form spectra from the integrated absorption spectra of neutral solutions. Therefore no experimental values of E and f are reported for these tautomers in Table 2. The ratio of tautomer (12) to tautomer (11) in solutions of neutral molecules of **IV** was determined by assuming that the ratios f_1/f_2 for the amino form (12) and the imino form (11) were the same as those for adenosine (6.8 [9]) and for inosine (0.7 [9]) respectively. Thus the ratio of tautomer (12) to tautomer (11) was found to be 7:3. It is difficult to carry out a similar calculation for cation **IV** as there is insufficient experimental data available to enable the tautomer bands to be separated.

The halfwidths of the absorption bands corresponding to separate electronic transitions in the ionic and tautomeric forms of natural purine and pyrimidine nucleic bases [9] and in most of the compounds investigated in this work do not generally exceed 5.5 - 6.0 kilokaysers. Exceptions are found in the spectrum of anion (7) and in the long wave bands of the absorption spectra of neutral aqueous solutions of **IIa**, **IIIa** and **IIIb**. The marked increase in the halfwidth of the longest absorption band of anion (7) is probably related to the transfer of a substantial portion of the π electron charge (more than 0.6 electron charge units) from the deprotonated oxygen and the exocyclic nitrogen atoms to the pyrimidine ring when the molecule undergoes a transition into the lowest excited singlet state. The abnormal width of the long wave band in neutral aqueous solutions of **IIa** appears to be due to the simultaneous presence of the neutral molecule (2) and the bipolar ion (4). Support for this proposal is given by the absence of broadening in neutral aqueous solutions of **IIb** and by the prediction of quantum chemical calculations that the longest absorption band will be markedly bathochromically shifted relative to the corresponding absorption band of the neutral molecule (3). As the substitution of the hydroxy group by the methoxy group does not produce significant spectral changes, it is probable that the absorption spectra of **IIb** are similar to that of **IIa** in form (2). Therefore the integrated absorption spectra of neutral aqueous solutions of **IIa** can be decomposed and the ratio of (2) to (4) determined as 3:7. It should be noted that, as in the case of the anion, some of the π electron charge was transferred from the oxygen attached to the N^4 atom to the

TABLE 2

Theoretical and experimental values of the energies for the electronic transitions in the ionic and tautomeric forms of N^4-methylcytidine and $N_{(3)}$-methylcytidine and their N^4-hydroxy and N^4-methoxy derivatives, the experimental oscillator strengths f, the radiation lifetimes τ_0 for the S_1 $\pi\pi^*$ state and the E values for low $n\pi^*$ transitions

	Cation (1)		*Neutral molecule*				*Anion (6)*		*Anion (7)*	
	E_1	E_2	*Amine (2)*		*Imine (3)*		E_1	E_2	E_1	E_2
			E_1	E_2	E_1	E_2				
N^4-methylcytidine										
PPP energy (eV)	4.31	5.85	4.41	5.44						
CNDO/S energy (eV)	4.54	6.19	4.57	5.77						
Experimental energy (eV)	4.42	5.71	4.58	5.37	—	—	—	—	—	—
f	0.59	0.58	0.52	0.44						
τ_0 (ns)	2.02	—	2.12	—						
$E_{n\pi^*}$	5.53	—	4.70	5.71						
$N_{(3)}$-methylcytidine										
PPP energy (eV)	4.31	5.85			4.93	5.64				
CNDO/S energy (eV)	4.54	6.19			4.83	5.95				
Experimental energy (eV)	4.46	5.69			4.68	5.61				
f	0.55	0.58	—	—	0.53	0.73	—	—	—	—
τ_0 (ns)	2.12	—			1.99	—				
$E_{n\pi^*}$	5.53	—			5.07	5.79				
N^4-hydroxycytidine (Ia)										
PPP energy (eV)	4.34	4.96	4.37	4.82	4.36	5.05	3.33	3.72	3.91	4.54
CNDO/S energy (eV)	4.52	5.70	4.58	5.53	4.70	5.68	4.10	4.79	3.79	4.76
Experimental energy (eV)	4.42	5.61	—	—	4.55	5.30	4.30	4.83	3.98	4.48[a]
f	0.61	0.45	—	—	0.33	0.70	0.20	0.53	0.73	0.10[a]
τ_0 (ns)	1.94	—	—	—	3.38	—	6.24	—	1.99	—
$E_{n\pi^*}$	5.59	6.52	4.75	5.72	4.95	5.90	4.36	5.24	4.26	5.60
N^4-methoxycytidine										
PPP energy (eV)	4.34	4.96	4.37	4.82	4.36	5.05	3.33	3.72		
CNDO/S energy (eV)	4.52	5.70	4.58	5.53	4.70	5.68	4.10	4.79		
Experimental energy (eV)	4.37	5.65	—	—	4.49	5.30	4.25	4.80	—	—
f	0.65	0.54	—	—	0.37	0.59	0.19	0.69		
τ_0 (ns)	1.86	—	—	—	3.10	—	6.73	—	—	—
$E_{n\pi^*}$	5.59	6.52	4.75	5.72	4.95	5.90	4.36	5.24		
N^4-methyl-N^4-hydroxycytidine (IIa)					*Bipolar ion (4)*					
PPP energy (eV)	4.34	4.96	4.37	4.82	4.07	4.90			3.91	4.54
CNDO/S energy (eV)	4.52	5.70	4.58	5.53	3.65	4.74			3.79	4.76
Experimental energy (eV)	4.30	5.45	4.45	5.50	4.04	4.90			3.94	4.65
f	0.78	0.17	0.58	0.20	0.70	0.59	—	—	0.61	0.22
τ_0 (ns)	1.53	—	2.01	—	2.02	—			2.39	—
$E_{n\pi^*}$	5.56	6.52	4.95	5.90	3.61	5.82			4.26	5.60
N^4-methyl-N^4-methoxycytidine (IIb)										
PPP energy (eV)	4.34	4.96	4.37	4.82						
CNDO/S energy (eV)	4.52	5.70	4.58	5.53						
Experimental energy (eV)	4.25	5.52	4.41	5.07	—	—	—	—	—	—
f	0.58	0.32	0.43	0.23						
τ_0 (ns)	2.32	—	2.58	—						
$E_{n\pi^*}$	5.56	6.52	4.95	5.90						

(continued)

TABLE 2 *(continued)*

	Cation (1)		*Neutral molecule*				*Anion (6)*		*Anion (7)*	
			Amine (2)		*Imine (3)*					
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
$N_{(3)}$-methyl-N^4-hydroxycytidine (IIIa)			*Anti isomer*		*Syn isomer*					
PPP energy (eV)	4.34	4.96	4.36	5.05	4.36	5.05				
CNDO/S energy (eV)	4.60	5.80	4.68	5.67	4.61	5.48				
Experimental energy (eV)	4.33	5.28	4.44	5.03	4.28	5.07	—	—	—	—
f	0.72	0.26	0.43	0.64	0.42	0.18				
τ_0 (ns)	2.15	—	2.73	—	3.00	—				
$E_{n\pi^*}$	5.68	6.62	4.74	5.89	5.01	5.78				
$N_{(3)}$-methyl-N^4-methoxycytidine (IIIb)										
PPP energy (eV)	4.34	4.96	4.36	5.05	4.36	5.05				
CNDO/S energy (eV)	4.60	5.80	4.68	5.67	4.61	5.48				
Experimental energy (eV)	4.29	5.31	4.41	4.98	4.27	5.05	—	—	—	—
f	0.54	0.15	0.27	0.62	0.31	0.14				
τ_0 (ns)	2.32	—	4.40	—	4.08	—				
$E_{n\pi^*}$	5.68	6.62	4.74	5.89	5.01	5.78				

[a]Unreliable data (see text for details).

TABLE 3

Theoretical and experimental values of the energies for the electronic transitions in the ionic and tautomeric forms of N^6-methoxyadenosine, the experimental oscillator strengths f, the radiation lifetimes τ_0 for the S_1 $\pi\pi^*$ state and the E values for low $n\pi^*$ transitions

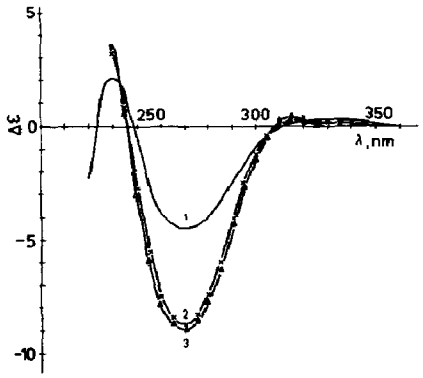
	Cation						*Neutral molecule*				*Anion (13)*	
	Structure (8)		*Imine (9)*		*Amine (10)*		*Imine (11)*		*Amine (12)*			
	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2	E_1	E_2
PPP energy (eV)	4.46	5.00	3.71	4.21	4.19	4.57	3.70	4.70	4.57	4.67	3.59	4.30
CNDO/S energy (eV)	4.49	4.79	3.93	4.31	4.60	5.06	3.59	4.96	4.89	5.03	4.02	4.59
Experimental energy (eV)	4.65[a]	5.57[a]	3.72	4.37	4.65[a]	5.57[a]	4.04	5.14[a]	4.64	5.14[a]	3.87	4.43
f	0.76[a]	0.19[a]	0.14	0.92	0.76[a]	0.19[a]	0.40	0.11[a]	1.04	0.11[a]	0.21	0.64
τ_0	1.41	—	9.92	—	1.41	—	3.54	—	1.03	—	7.34	—
$E_{n\pi^*}$	5.03	5.47	4.69	5.58	4.90	5.47	4.90	5.10	4.83	5.40	4.43	4.91

[a]Data compared with values calculated for several tautomers.

pyrimidine ring. However, the charge transfer is markedly less [26] than that occurring during the excitation of anion (7), and the halfwidth of the longest absorption band of the bipolar ion is about 5 kilokaysers, *i.e.* the value characteristic of pyrimidine and purine components [9].

3.3. *Syn–anti isomerism of N^4-hydroxy-$N_{(3)}$-methylcytidine and N^4-methoxy-$N_{(3)}$-methylcytidine*

We now consider the absorption spectra of neutral aqueous solutions of **IIIa** and **IIIb**. The formation of bipolar ions similar to (4) is almost impossible in these compounds. It is possible to propose a structure for **IIIa** with the formal positive sign at the $N_{(3)}$ atom, an $N_{(3)}=C_{(4)}$ double bond, a $C_{(4)}—N^4$ single bond and a proton localized at N^4. However, this structure for **IIIa** appears to be unlikely as its spectroscopic and photochemical behaviour is almost identical with that of **IIIb** where such a structure is impossible. No substantial intramolecular transfers of π electron charge occur on the transition of these molecules into the lowest excited state. Therefore the explanation proposed above for absorption band broadening is not valid in this case.



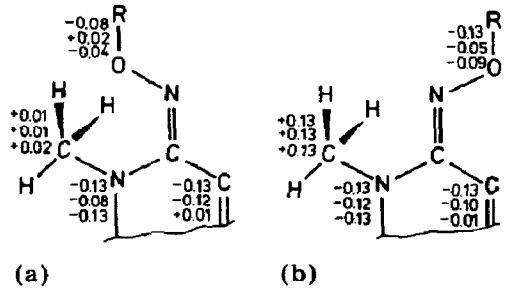


Fig. 8. CD spectra of alkaline solutions of $N_{(3)}$-methyl-N^4-methoxycytidine (incident light intensity, 4×10^{15} quanta cm^{-2} s^{-1}; λ = 313 nm): curve 1, before irradiation; curve 2, after the absorption of 1 quantum per nucleoside; curve 3, after the absorption of 7 quanta per nucleoside.

Fig. 9. Part of the molecular diagram for (a) *syn* and (b) *anti* isomers of $N_{(3)}$-methyl-N^4-methoxycytidine. The groups of numbers give the charges on the atoms of the molecule in the S_0, S_1 and T_1 states (reading from top to bottom).

The most probable reason for the abnormal width of the longest band in the absorption spectra of these compounds is the presence in the solution of alternative structures with various absorption spectra. The existence of more than one band in the long wave region (above 250 nm) of the CD spectra (Fig. 8) confirms this hypothesis. As compounds **III** are fixed tautomers, the most probable explanation for the broadening of the first band in the spectrum is the simultaneous presence in the aqueous solution of the *syn* and *anti* isomers of these compounds which have different absorption spectra (Table 2).

Quantum chemical calculations using the CNDO/S approximation predict that the first absorption band of the *syn* isomer is at a longer wavelength than that of the *anti* isomer. The molecular diagram given in Fig. 9 shows that the negatively charged oxygen atom of the N—O group of the *anti* isomer of III in the ground state S_0 is located close to the negatively charged $C_{(5)}$. In the *syn* isomer this oxygen atom is adjacent to the positively charged methyl group at $N_{(3)}$. The structure of the *syn* isomer should apparently be stabilized by non-valent interactions of the oxygen atom of the hydroxy or methoxy group with hydrogen atoms of the methyl group. This fact together with the observed transformation of the starting absorption spectrum to a shorter wavelength upon irradiation (see below) suggests that the *syn* isomer is dominant in the mixture of isomers in the ground state. Since the C_4—N^4 bond is double in the neutral molecules of the compounds investigated (the order P_{S_0} of this bond is 0.84 [15, 26]), the possibility of rotation around it should be excluded. Therefore the dark equilibrium for the isomer mixture is most likely to proceed solely via the cation with substantially lower $C_{(4)}$—N^4 bond order (P_{S_0} = 0.58 [16, 26]).

When the *syn* isomer is excited the $C_{(4)}$—N^4 bond in the S_1 and T_1 states (P_{S_1} = 0.65, P_{T_1} = 0.59 [26]) weakens and the charges at the atoms participating in the isomer stabilization change markedly. Thus the charge at the oxygen atom of the N—O group becomes positive on transition to S_1, whereas the signs of the charges at $C_{(5)}$ and $N_{(3)}$—CH_3 are almost constant. On transition into the T_1 state the charges at $N_{(3)}$—CH_3 and at the oxygen atom of the N—O group change only slightly, whereas the charge on $C_{(5)}$ becomes positive. Therefore quantum chemical calculations predict that the equilibrium between the *syn* and the *anti* isomers should be markedly shifted towards the *anti* isomers on excitation.

It should be stressed, however, that the photoinduced *anti–syn* reaction would also occur on excitation of *anti* isomers, as can be seen from the molecular diagram. Therefore when the isomer mixture is irradiated under conditions in which "dark" isomerism (via the cation) can be neglected, a "light" equilibrium which is distinct from the dark equilibrium should be established.

It is evident that if the UV absorption spectra of the *syn* and *anti* isomers are different, the ratio of isomers in the light equilibrium must depend on the wavelength of the incident light. If the ratios of the isomers in the light and the dark equilibria are different, the primary rate of restoration of the dark equilibrium after irradiation must be proportional to the concentration of the III^+H cations, *i.e.* at pH $\gg$ pK_1 it must be proportional to the concentration of the hydrogen ions.

To check our assumptions and the theoretical predictions a combined investigation of the absorption, CD and PMR spectra of the compounds during irradiation and after subsequent storage in darkness was carried out.

The UV irradiation of alcoholic or alkaline (pH $\geqslant$ 9) aqueous solutions of IIIa and IIIb produces major changes in their absorption (Fig. 10) and CD (Fig. 8) spectra. Complete restoration of the original absorption and CD

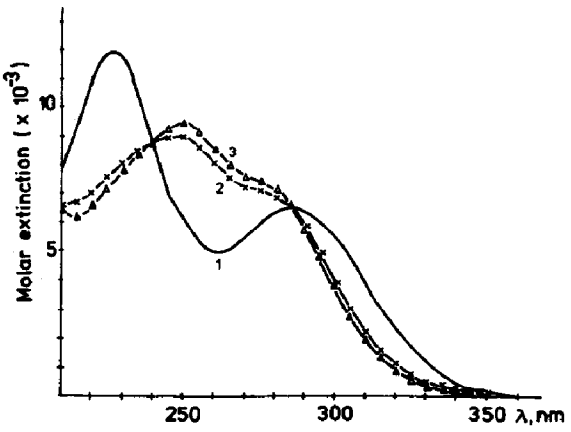


Fig. 10. UV absorption spectra of alkaline solutions of $N_{(3)}$-methyl-N^4-methoxycytidine during irradiation under the conditions given in Fig. 8: curve 1, before irradiation; curve 2, after the absorption of 1 quantum per nucleoside; curve 3, after the absorption of 7 quanta per nucleoside.

spectra occurs on cessation of irradiation (similar results are obtained for the PMR spectra (see below)). The spectra measured during and after irradiation have well-defined isosbestic points, which indicate that all spectral transitions occurring on irradiation result from the transformation of one component of the solution into another and that all transitions occurring after irradiation result from the reversal of these transformations. The absorption spectra of both components were calculated using the procedure described in Section 2 (Fig. 6). One component was found to be dominant in the starting solutions of **IIIa** and **IIIb** (for example, the concentration of the major component of the **IIIb** solution was 95%) and under irradiation the equilibrium shifted towards the minor component. The light equilibrium was achieved when the ratio of the first to the second component was 1:6 for λ_{irr} = 254 nm and 1:3 for λ_{irr} = 313 nm. Since the observed spectroscopic and photochemical behaviours of **IIIa** and **IIIb** are very similar, the PMR spectra of the irradiated solutions were investigated for **IIIb** only.

In the PMR spectra of unirradiated solutions of **IIIb** one series of signals was observed from the protons $H^{(5)}$, $H^{(6)}$ and $H^{(1')}$ and from the protons of the CH_3 group at $N_{(3)}$ with an intensity ratio of 1:1:1:3 at pD values from 6 to 11 (Fig. 11 and Table 4). The absence of the second series of signals, in view of the moderate solubility of **IIIb** in D_2O, is in complete agreement with the analysis of the UV absorption spectra which indicates that the content of the second component is less than 5%. As the irradiation of the alkaline aqueous solutions proceeds, the PMR spectra of **IIIb** show a decrease in the signal intensity of the initial spectrum and a proportional increase in the intensity of a new series of signals. All the changes in the PMR spectra occur concomitantly with the changes in the UV and CD spectra discussed above. When light equilibrium is achieved the changes in the spectra cease.

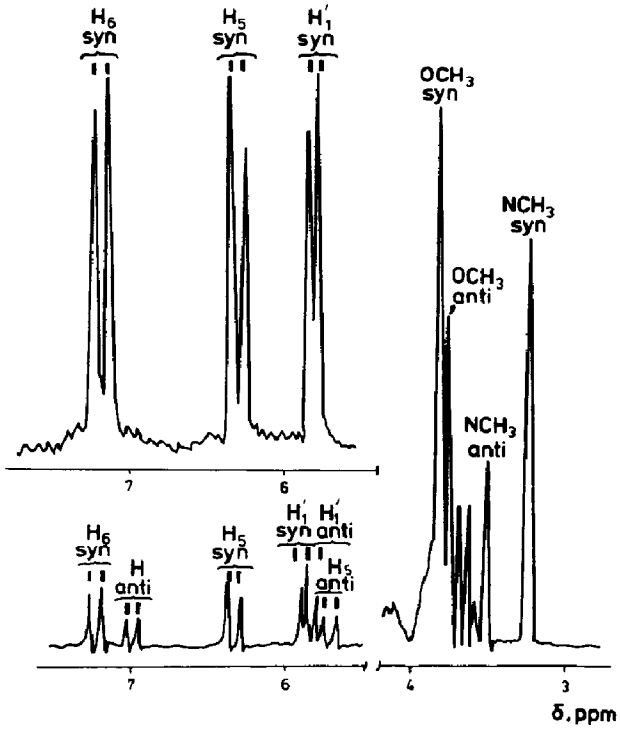


Fig. 11. PMR spectra of $N_{(3)}$-methyl-N^4-methoxycytidine in D_2O (pD 11.0) prior to irradiation (upper spectrum) and after the absorption of 7 quanta per nucleoside (λ = 313 nm) (lower spectrum).

Thus the analysis of the CD, PMR and absorption spectra shows that the starting solutions of **IIIa** and **IIIb** are composed of two components, one of which is dominant. When the solutions are irradiated the equilibrium is shifted towards the minor component and after irradiation the initial dark equilibrium is restored. Hence the experimental photochemical and subsequent dark behaviour of compounds **IIIa** and **IIIb** is in complete agreement with the theoretical predictions for the photoinduced and dark reactions of *syn–anti* and *anti–syn* transformations. Comparison of the experimental and theoretical data (Figs. 6 and 9 and Table 2) enables the major component in the dark equilibrium to be identified as the *syn* isomer and the minor component to be identified as the *anti* isomer.

The preponderance of the *syn* isomer of **IIIb** in the unirradiated solution was experimentally confirmed by PMR spectroscopy. The orientation of the methoxy group relative to $H^{(5)}$ in the isomers of **IIIb** was determined by measuring the NOE for $H^{(5)}$. The effects of the methoxy group protons were suppressed. The NOE value did not exceed 4% in the initial alkaline solution of **IIIa**. However, an increase in the content of the second isomer up to about 75% on irradiation resulted in an NOE value of 10% ± 4%, indicating a decrease in the distance between the methoxy group and $H^{(5)}$. Thus, in accordance with the theoretical predictions, the dominant isomer in the starting solution of **IIIb** is the *syn* form but the percentage of *anti*

TABLE 4

Chemical shifts of the proton magnetic resonance spectra of cytidine, N^4-hydroxycytidine and their methyl derivatives

Nucleoside	pD	$H^{(5)}$[a]	$H^{(6)}$[a]	$H^{(1')}$[a]	$N_{(3)}CH_3$[b]	N^4CH_3[b]	OCH_3[b]
Cytidine	2	6.24	8.12	5.88	—	—	—
	7	6.03	7.80	5.88	—	—	—
$N_{(3)}$-methylcytidine	7	6.35	8.17	5.92	3.48	—	—
	12	5.92	7.26	5.89	3.24	—	—
N^4-methylcytidine	7	6.01	7.72	5.95	—	2.92	—
N^4-hydroxycytidine (Ia)	1	6.13	7.98	5.88	—	—	—
	7	5.79	7.12	5.90	—	—	—
N^4-methoxycytidine (Ib)	1	6.00	7.75	5.85	—	—	3.89
	7	5.77	7.14	5.88	—	—	3.83
N^4-methyl-N^4-hydroxycytidine (IIa)	7	6.30	7.59	5.91	—	3.51	—
N^4-methyl-N^4-methoxycytidine (IIb)	7	6.42	7.98	5.93	—	3.40	3.81
$N_{(3)}$-methyl-N^4-hydroxycytidine (IIIa)	7	6.33	7.18	5.88	3.20	—	—
$N_{(3)}$-methyl-N^4-methoxycytidine (IIIb)	7	6.34	7.23	5.88	3.21	—	3.78
	11	6.34	7.23	5.88	3.21	—	3.78
	11[c]	5.77	7.00	5.83	3.49	—	3.75

[a] Doublet.
[b] Singlet.
[c] This series of signals is produced by the UV irradiation of alkaline solutions.

isomer increases on irradiation. The similarity of the chemical shifts of the resonances of $H^{(5)}$, $H^{(6)}$ and $H^{(1')}$ and of the CH_3 protons at $N_{(3)}$ in the initial IIIa and IIIb solutions to the chemical shifts observed for the isomers on UV irradiation in alkaline solutions (Table 4) suggests that in the dark equilibrium state IIIa is mainly in the *syn* form and that the spectral changes occurring on irradiation are due to an increase in the content of *anti* isomer.

The transition from the *syn* to the *anti* isomer observed for the neutral forms of compound III which are fixed imino tautomers is accompanied by changes in the chemical shifts of the $H^{(5)}$ resonances from approximately 6.3 to 5.8 ppm. As the insertion of methyl groups does not produce significant changes in the electronic structure, it can be assumed that these values of the chemical shift of the $H^{(5)}$ resonances are characteristic of the *syn* and *anti* isomers of neutral imino tautomers of other substituted N^4-hydroxycytidines. On these grounds it is expected that N^4-hydroxycytidine, N^4-methoxycytidine and $N_{(1)}$-methyl-N^4-hydroxycytosine with chemical shifts for the $H^{(5)}$ resonances of 5.79 ppm (Table 4), 5.77 ppm (Table 4) and 5.69 ppm [28] respectively are in the *anti* isomer conformation in solution and that $N_{(1)}$,$N_{(3)}$-dimethyl-N^4-hydroxycytosine with a chemical shift for the $H^{(5)}$ resonance of 6.15 ppm [28] is in the *syn* isomer conformation. However, Brown *et al.* [28] reached the opposite conclusion and assigned $N_{(1)}$-methyl-N^4-hydroxycytosine and its $N_{(3)}$-methyl derivative to the *syn* and *anti* conformations respectively [28]. This conclusion was based on the

suggestion that the *syn* isomer of the first compound is stabilized in an aqueous solution by the hydrogen bond between $N_{(3)}H$ and the oxygen atom of the N^4OH group, whereas such a stabilization is impossible for the second compound and, additionally, the methyl group at $N_{(3)}$ sterically hinders the *syn* location of the N^4OH group. However, the steric position of $N_{(3)}H$ and the N^4OH group in N^4-hydroxycytosine is unfavourable for hydrogen bonding (the distance between $N_{(3)}H$ and O is 2.17 Å and the angle between $N_{(3)}H$ and O is 100° [15]), but the methyl group at $N_{(3)}$ does not sterically hinder the *syn* position of the N—O bond as is proved by the existence of both isomers for compounds **IIIa** and **IIIb**. Moreover, the chemical shifts of the $H^{(5)}$ resonances demonstrate that *syn* isomers are dominant in solutions of **IIIa**, **IIIb** and $N_{(1)}$,$N_{(3)}$-dimethyl-N^4-hydroxycytosine and that *anti* isomers are dominant in solutions of **Ia** and **Ib**, *i.e.* the presence of the methyl group at $N_{(3)}$ favours the stabilization of the *syn* isomer. The use of X-ray data to predict the isomerism of N^4-hydroxycytosine and its derivatives in the solution was also insufficiently rigorous. For example, although $N_{(1)}$,$N_{(3)}$-dimethyl-N^4-hydroxycytosine and $N_{(1)}$-methyl-N^4-hydroxycytosine hydrochloride exist in the crystalline form as *syn* isomers [15, 16], the *syn* isomer of the first compound is stabilized in the crystal by an intermolecular hydrogen bond, which is unlikely to be the case in solution, and the ionization of the second compound from its neutral form to the cation leads to significant changes in the charges at the $N_{(3)}$ and $C_{(5)}$ atoms that should favour the stabilization of the *syn* isomer hydrochloride. When the neutral forms of **Ia** and **Ib** are ionized to cations the changes in the chemical shifts of the $H^{(5)}$ resonances are in agreement with those expected for a transformation from the *anti* to the *syn* isomer. A similar increase in the chemical shifts is also observed when cytidine and $N_{(3)}$-methylcytidine are protonated (Table 4). Therefore the chemical shift of the $H^{(5)}$ resonance can only be used to characterize the isomers of neutral imino tautomers of N^4-hydroxycytosine derivatives.

The photoinitiated *syn–anti* isomerization and the dark *anti–syn* transformation of the neutral imino forms of compounds **IIIa** and **IIIb** including acid–base equilibria are shown in Fig. 12. It should be emphasized that both the dark and the light equilibria between *syn* and *anti* isomers are dynamic and depend on various external factors such as the wavelength of the exciting light and the pH value.

The quantum yields of the photoinitiated and the rate constants of the dark *syn–anti* and *anti–syn* isomerization reactions were calculated using the

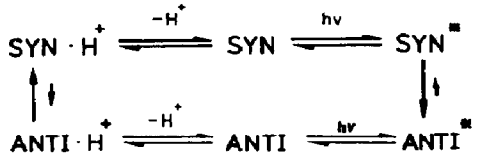


Fig. 12. *Syn–anti* photoisomerization and the dark *anti–syn* transformation for $N_{(3)}$-methyl-N^4-methoxycytidine.

TABLE 5

Quantum yields and rate constants at 37 °C of the photoinduced and spontaneous *syn–anti* isomerization of fixed imino tautomers of N^4-hydroxycytidine and N^4-methoxycytidine

Nucleoside	*Solvent*	ϕ_{sa}	ϕ_{as}	K_{as}[a] (s^{-1})	K_{sa}[a] (s^{-1})	K_{as}'[b] (s^{-1})	K_{sa}'[b] (s^{-1})
$N_{(3)}$-methyl-N^4-hydroxycytidine (IIIa)	Water	0.05	0.07	3.9×10^{-3} (8.9)	1.95×10^{-4} (8.9)	2.7×10^{5}	1.35×10^{4}
	Ethanol	0.10	0.15	1.5×10^{-2}	—		
$N_{(3)}$-methyl-N^4-methoxycytidine (IIIb)	Water	0.15	0.26	4.5×10^{-4} (8.9) 3.5×10^{-6} (11)	2.25×10^{-5} (8.9) 1.75×10^{-7} (11)	2.2×10^{4}	1.1×10^{3}
	Ethanol	0.14	0.23	3.2×10^{-3}	—		

[a] The constants are calculated from the changes observed in the spectra of alkaline solutions after the cessation of irradiation; the pH values are given in parentheses.
[b] Rate constants for cation isomerization.

formulae given in Section 2 and are presented in Table 5. The quantum yields of the photoinitiated isomerization reactions are almost two orders of magnitude higher than that of the photoinduced rupture of the N—O bond [4]. Despite the fact that ϕ_{as} is greater than ϕ_{sa} a shift in the equilibrium between the *syn* and the *anti* isomers in the direction of the latter is observed when solutions of **IIIa** and **IIIb** are irradiated and is related to the relatively low concentration of *anti* isomers in the initial unirradiated solutions. The rate constants of the dark reactions are proportional to the concentration of hydrogen ions in the solution. This is in agreement with the assumption that the dark isomerization of these compounds proceeds via the cation.

4. Conclusion

Although $n\pi^*$ transitions were not observed for the compounds investigated, the quantum chemical calculations using the CNDO/S approximation indicate that for all the ionic and tautomeric species of these compounds the $n\pi^*$ transitions are likely to be at shorter wavelengths than the first $\pi\pi^*$ transitions. Exceptions are the bipolar ion (4) and the neutral amino form (12) for which the calculation predicts that the $n\pi^*$ transitions are at a longer wavelength.

The investigations reported here of the spectral characteristics of the ionic and tautomeric forms of the molecules and of the photoinitiated *syn*–*anti* isomerization reactions is a specific but significant demonstration of the efficiency of the combined experimental and theoretical approach developed by us earlier [8, 9] for investigations of the physicochemical, spectroscopic and photochemical properties of a series of related compounds. This complex approach is based on a combination of spectroscopic, mathematical (computer analysis of spectral data) [8, 9] and quantum chemical [9] methods. In particular, the decomposition of the absorption spectra into bands corresponding to separate electronic transitions is almost impossible without the corresponding quantum chemical calculations which enable the number of bands and their approximate parameters to be determined. A deviation of more than 0.3 eV between experiment and theory in the determination of the first electronic transition means that one of the ionic or tautomeric species can be practically excluded from consideration. Finally, in order to carry out a mathematical decomposition of the bands the ranges of parameters characteristic for the class or the group of compounds under investigation must be known. A knowledge of the electronic structures of the compounds studied in the ground state and in the excited singlet and triplet states allows the potentially reactive and photoreactive centres of the molecules to be determined and the effect on the spectral properties of changes in the electronic structure produced by excitation of the molecule to be followed [9, 26]. Such an approach significantly increases the efficiency of the investigations and enables experiments to be planned more accurately.

Acknowledgment

We wish to express our sincere thanks to Dr. G. I. Yakovlev, Institute of Molecular Biology, U.S.S.R. Academy of Sciences, for helpful discussions of the results of the PMR investigations.

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