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INVESTIGATION OF THE CIRCULAR DICHROMISM SPECTRA OF BROMINE-SUBSTITUTED NUCLEIC ACID FRAGMENTS

II. CIRCULAR DICHROISM SPECTRA OF 5-BROMINE-SUBSTITUTED PYRIMIDINE NUCLEOTIDES

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In the present paper we describe the results of a further study of the circular dichroism (CD) spectra of bromine-substituted fragments of nucleic acids [1]. We have obtained the CD spectra of 5-bromine-substituted nucleotides of uracil and cytosine — the ribo and 2'-deoxyribonucleoside 5'-monophosphates — over a wide pH range and also in methanol. Analysis of the results obtained consist mainly in a comparison of the changes in the CD Spectra with the conformational rearrangements of the nucleotides investigated.

Figure 1 gives the CD spectra of 5-bromouridine 5'-monophosphate (5-mono-UMP) and of 5-bromo-2'-deoxyuridine 5'-monophosphate (5-bromo-dUMP), and Fig. 2 gives the CD spectra of 5-bromocytidine 5'-monophosphate (5-bromo-CMP; and of 5-bromo-2'-deoxycytidine 5'-monophosphate (5-bromo-dCMP). All the nucleotides were in the form of the disodium salts. Table 1 gives their spectral properties.

In the wavelength range of 200-300 nm three Cotton effects (CEs) were recorded which corresponded to π—π^* transitions in the B_{2u}, B_{1u}, and E_{1ua} bands (localized, respectively, in the 280-300, 240-260 and 220-235 nm region). The spectra of the compounds studied showed the features that have been observed in the CD spectra of the unbrominated nucleotides. In particular, the maximum of the B_{2u} CE band of 5-bromo-UMP and of 5-bromo-dUMP shifts in the long-wave direction from the absorption maximum, while the B_{1u} CE has the opposite sign to that of

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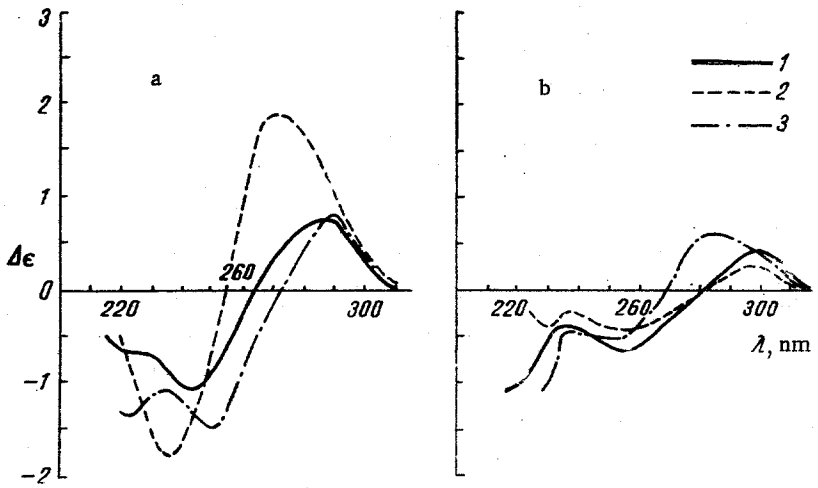

Fig. 1. CD spectra of 5-bromo-UMP (a) and of 5-bromo-dUMP (b): 1) pH 2 and 7; 2) pH 12; 3) methanol.

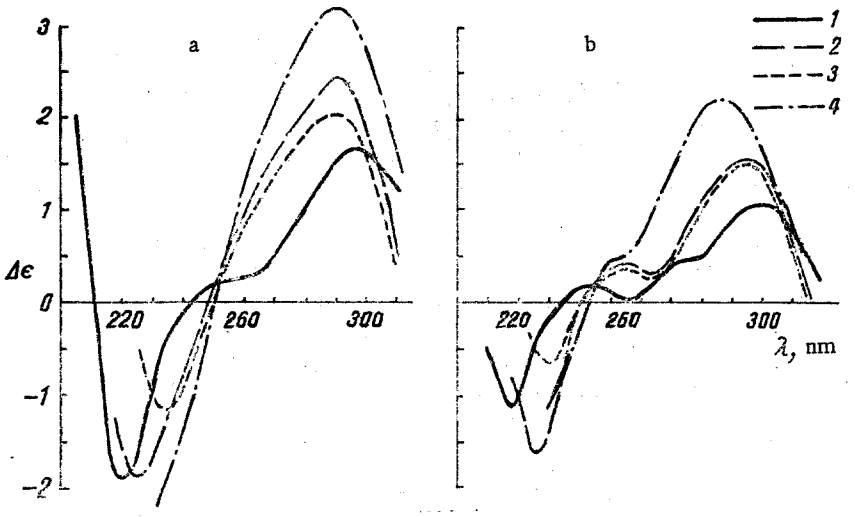

Fig. 2. CD spectra of 5-bromo-CMP (a) and of 5-bromo-dCMP (b): 1) pH 2; 2) pH 7; 3) pH 12; 4) methanol.

the B_{2u} band. This can be seen from a comparison of the UV and CD spectral characteristics of these compounds (Table 1). This characteristic has also been observed for all the unbrominated uridine analogs investigated [2].

However, as can be seen from Table 1, the maxima of the B_{2u} CE and of the UV spectrum of 5-bromo-UMP at pH 12 practically coincide. One of the reasons for this may be the protonation of the uracil nucleus. It has been shown [2] in a study of the influence of solvents on the spectra of uridine derivatives that the observed shifts in the CD bands are due to the formation of hydrogen bonds with the participation of the ketonic oxygen and are due to π—π^* transitions. On this basis, the authors concerned drew the conclusion that if the change in the distribution of the charges of the uracil nucleus due to π—π^* excitation decreases the negative charge on the ketonic oxygen atom bound by a hydrogen bond with water, a blue shift will be expected relative to the initial state, which is characterized by the absence of such a bond. This makes understandable the fact that in methanol all the CE bands in the spectrum of 5-bromo-UMP are shifted in the red direction (Table 1) as compared with the positions of the corresponding extrema in aqueous solutions. In actual fact, methanol as a solvent possesses a lower hydrogen-acceptor strength than water, and therefore the partial negative charge on ketonic oxygen atoms bound by a hydrogen bond with methanol will be greater than on ketonic oxygen atoms bound by a hydrogen bond with water.

TABLE 1. Spectral Properties of 5-Bromine-Substituted Pyrimidine Nucleotide

Compound	Medium	UV spectrum		CD spectrum		
		λ_{max}, nm ($\varepsilon \times 10^{-3}$)	λ_{min}, nm	λ_{extr}, nm ($\Delta\varepsilon$)		
				E_{1u}	B_{1u}	B_{2u}
5-Bromo-UMP	pH 2	280 (8,1)	243	220 (—0,70)	246 (—1,15)	286 (0,75)
	pH 7	279 (7,9)	245	220 (—0,70)	246 (—1,45)	286 (0,75)
	pH 12	278 (5,8)	251		234 (—1,80)	275 (1,80)
	Methanol	280 (8,0)	246	223 (—1,35)	250 (—1,55)	290 (0,83)
5-Bromo-deoxy-UMP	pH 2	280 (8,1)	242	237 (—0,35)	256 (—0,70)	298 (0,40)
	pH 7	280 (7,9)	245	237 (—0,35)	256 (—0,70)	298 (0,40)
	pH 12	278 (5,8)	251	229 (—0,40)	256 (—0,45)	295 (0,25)
	Methanol	279 (7,8)	244	235 (—0,45)	256 (—0,50)	287 (0,58)
5-Bromo-CMP	pH 2	300 (10,2)	255	220 (—1,90)	Positive shoulder	298 (1,60)
	pH 7	290 (7,5)	262	225 (—1,90)	. . . . .	290 (2,40)
	pH 12	290 (7,6)	262	242 (—1,20)	. . . . .	290 (2,10)
	Methanol	290 (7,7)	262	225 (—3,0)	. . . . .	290 (3,14)
5-Bromo-deoxy-CMP	pH 2	298 (6,4)	254	217 (—1,1)	245 (0,15)	300 (1,30)
	pH 7	288 (4,8)	260	226 (—1,70)	254 (0,40)	295 (1,50)
	pH 12	288 (4,9)	260	232 (—0,90)	254 (0,40)	295 (1,55)
	Methanol	288 (4,9)	260	. . . . .	Positive shoulder	285 (2,16)

The participation of the oxygen of the 2-oxo group in the formation of a hydrogen bond with the solvent is unlikely. It is known [3] that in the uridine molecule a fairly strong intramolecular hydrogen bond with the participation of the 2-oxo oxygen atom and the 2'hydroxy group of the ribose exists. The similarity of the CD spectra of brominated and unbrominated uridine analogs permits the conclusion that such an intramolecular hydrogen bond also exists in the 5-bromo-UMP molecule. The absence of this bond in the 5-bromo-dUMP molecule probably leads to the characteristic changes in the CD spectra. In actual fact, in the 5-bromo-dUMP molecule there are two ketonic oxygen atoms that can form hydrogen bonds with the solvent. The replacement of the stronger intramolecular hydrogen bond with the participation of the 2-oxo oxygen atom in the molecule of 5-bromo-UMP by the weaker hydrogen bond with water in the 5-bromo-dUMP molecule apparently leads to the situation that all the CD bands in the latter case are shifted in the red direction. This can be seen if we compare the positions of the maxima of the CD bands of aqueous solutions of 5-bromo-UMP and of 5-bromo-dUMP, which are given in Table 1.

The ionization of the heterocyclic base in an alkaline medium (pH 12) apparently leads to a strengthening of the hydrogen bond between the 2-oxo oxygen atom and the 2'-hydroxyl of the ribose in the 5-bromo-UMP molecule. This appears in the CD spectrum of the latter (Fig. 1a, curve 2) in the form of an increase in the amplitude and a fairly blue shift of the B_{2u} bands in other media.

For 5-bromo-dUMP, no such pH dependence of the positions of the maxima in the CD spectrum is observed, which is evidence in favor of the considerations given above. In addition to this, the absence of such an intramolecular hydrogen bond in 5-bromo-dUMP is responsible for the fact that $\Delta\varepsilon$ for the B_{2u} band of the latter is smaller than $\Delta\varepsilon$ of the analogous band for the ribo derivative (Table 1). The 5-bromo-dUMP molecules are probably conformationally more mobile in solution than the 5-bromo-UMP molecules.

It follows from this that, particularly in an alkaline medium (pH 12), the 5-bromo-UMP molecule exists in a folded conformation which acquires rigidity through an increase in the strength of the intramolecular hydrogen bond. This conformational rearrangement is probably accompanied by a decrease in the size of the dihedral angle between the planes of the heterocyclic base and of the sugar residue. We have also observed such a conformational rearrangement in the case of UMP when measuring the CD spectra of the latter in an alkaline medium. In these circumstances, a small stepwise increase in pH leads to a very rapid increase in the amplitude of the B_{2u} band. As our investigations have shown, this rearrangement is localized at the pH point equal to the pK value of the heterocyclic base (9.5 for UMP) and takes place in a time comparable with the recording time of the instrument (10 sec even at 0°C). We have found no description of such a transition of the conformational type in the literature. A detailed investigation of the kinetics of this process requires further study using faster methods.

The CD spectra of 5-bromo-CMP (Fig. 2a) and 5-bromo-dCMP (Fig. 2b) have large positive B_{2u} CEs, and the B_{1u} band is observed as a small positive perturbation in the 240-260 nm region (Table 1). For 5-bromo-dCMP the B_{1u} CE is more pronounced. Probably, in the CD spectrum of 5-bromo-CMP the B_{1u} band is masked by the stronger B_{2u} and E_{1ua} bands. In methanol, (Fig. 2, curve 4) the amplitudes of these bands rise but, as can be seen from Table 1, their positions scarcely change. The interaction due to hydrogen bonds must cause the characteristic blue shift of the maxima of these bands. Consequently, the corresponding changes in the CD spectra may be assigned to the purely conformational type. The change in the CD profile in an acid medium is probably connected with protonation of the heterocyclic base (pK 4.5). The maximum of the absorption spectra and the B_{2u} CE coincide. This also permits the changes in the CD curves to be connected with conformational rearrangements of the compounds investigated.

Just as in the case of the uridine derivatives (Fig. 1), all the maxima of the B_{2u} CD bands of 5-bromo-dCMP are shifted in the red direction as compared with the positions of the analogous maxima in the CD spectra of 5-bromo-CMP (Fig. 2a). Consequently, an intramolecular hydrogen bond with the participation of the 2-oxo oxygen atom and the 2'-hydroxyl of the ribose also exists in the 5-bromo-CMP molecule for the same reasons that were discussed above in connection with the analogous changes in the CD spectra of the uridine nucleotides.

The question of precisely what conformational changes take place with the pyrimidine derivatives in solution and the connection of these changes with optical activity requires further refinement. In the paper of Miles et al. [4], the molar ellipticity of the B_{2u} CE is considered as a function of the angle of rotation φ_{CN} in the form of the corresponding diagram. According to these ideas, and also taking into account the far-reaching analogy in the CD spectra of the brominated and unbrominated nucleotides, we explain the CD spectra obtained by the existence of the anti conformation of the compounds investigated. The same intramolecular forces are involved in the stabilization of this conformation as in the case of the unbrominated compounds. Thus, the halogenation of the pyrimidine nucleotides in position 5 causes no fundamental change of the conformation in aqueous and alcoholic solutions.

EXPERIMENTAL

The methods and instruments used for investigating the CD spectra of 5-bromine-substituted pyrimidine nucleotides were similar to those described in the preceding paper [1].

SUMMARY

The CD spectra of 5-bromine-substituted derivatives of nucelotides of uracil and cytosine, namely the ribo- and 2'-deoxyribonucleoside 5'-monophosphates, have been obtained in neutral, acid, alkaline, and alcoholic solutions. Three CEs have been recorded in the 220-300 nm region, corresponding to $\pi-\pi^*$ transitions in the B_{2u}, B_{1u}, and E_{1ua} bands. It has been concluded that an intramolecular hydrogen bond exists between O-2 and the 2'-hydroxyl in the 5-bromine-substituted ribonucleotides of uridine and cytidine. A transition of the conformational type localized at a pH equal to the pK value of the heterocyclic base has been detected for 5-bromo-UMP and UMP. On comparing the main characteristic of the CD spectra of the compounds investigated with the spectra of the unbrominated analogs given in the literature, it has been concluded that the halogenation of a pyrimidine base in position 5 does not fundamentally change the ratio of the most probable rotational stereomers, and the compounds investigated have the anti conformation in solution.

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