Chemistry

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Fig. 1

Figure 1: Fig. 1

Abstract

Full Text

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The Action of Bromine on the Optical Properties of Purine and Pyrimidine Bases

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Bromination for the identification of uracil and cytosine was first used by Wheeler and Johnson ([1]) as early as 1907. Subsequently, various color reactions for pyrimidines using bromine were proposed ([2-5]). The absorption spectra of brominated pyrimidine bases and their nucleosides were described by Suzuki and Ito ([6]); a brief report on the bromination of nucleotides was made by Jones and Woodhouse ([7]). In studying oligonucleotides of a ribonuclease hydrolysate of yeast transfer RNA, we made extensive use of their spectra and systematically applied bromination to identify the base composition ([1-7]). We described ([8], [9]) the effect of bromination on the spectra of oligoribonucleotides and ribonucleotides.

Fig. 1. Spectrum of uracil. **1** —unbrominated at pH 2; **2** —brominated, bromine water 1 : 20; **3** —brominated, bromine water 1 : 5; **4** —bromine water saturated; **5** —5-bromouracil from the chromatogram; **6** —authentic 5-bromouracil

The subject of the present communication is the question of the mechanism of changes in the spectra of nitrogenous bases during bromination.

Bromination was carried out as follows: to 3 ml of an approximately $1.0 \cdot 10^{-4}$ M solution of the base in 0.1N HCl, 0.1 ml of saturated bromine water, or bromine water diluted 5- and 20-fold, was added and the mixture was left for 10 min at room temperature. It was then aerated for 10 min, and the spectrum was recorded on a Hitachi recording spectrophotometer in the region 220–340 mμ. In kinetic determinations, unreacted bromine was first extracted with chloroform, and the solution was then aerated.

Paper chromatography of bromo derivatives of nitrogenous bases was carried out on Leningrad "slow" paper in an ethanol—acetate-ammonia buffer system, pH 7.5 (70 : 30).

Upon bromination of uracil, the absorption band with a maximum at 260 mμ disappears and the spectrum takes the form shown in Fig. 1. When bromine

Fig. 2. Spectrum of cytosine. Designations 1–4 are the same as in Fig. 1

Figure 2: Fig. 2. Spectrum of cytosine. Designations 1–4 are the same as in Fig. 1

water acts on uracil, 5,5-dibromo-6-hydroxyhydrouracil (I) is formed.

(I) (II)

[structural formulas shown in the original]

The mechanism of this reaction [10] is as follows. The active agent of bromine water is primarily BrOH in the form of Br^+ and OH^- ions. First, BrOH adds at the 4,5 bond with saturation of the double bond; then a molecule of H_2O is eliminated and 5-bromouracil I is obtained (with restoration of the double bond); after this, one more molecule of BrOH adds and 5,5-dibromo-6-oxyhydrouracil II is formed.

Fig. 2. Spectrum of cytosine. Designations 1–4 are the same as in Fig. 1.

From dibromouracil, under comparatively mild conditions (for example, on heating in an acidic medium), monobromouracil can be obtained. On chromatography of dibromouracil in the ethanol–acetate–ammonia buffer system, pH 7.5, it is converted into a derivative with the spectrum shown in Fig. 1, 5. This is 5-bromouracil, whose authentic spectrum (Fig. 1, 6) has the same maximum at 275 mμ. Repeated bromination of the 5-bromouracil extracted from the chromatogram again converts it into dibromouracil.

On bromination of cytosine (Fig. 2), the absorption band with a maximum at 280 mμ disappears and a new small maximum appears at 296 mμ, especially distinct when bromine water 1 : 20 is used. This maximum is constant and can serve for identification of cytosine derivatives. The question of the products of bromination of cytosine is not clear. Wheeler and Johnson [1], brominating cytosine, obtained 5,5-dibromo-6-oxyhydrouracil, which with appropriate treatment can be converted into monobromouracil (II). Using paper chromatography we found that, under the bromination conditions employed in the present study, cytosine gives neither dibromouracil nor monobromouracil, but an unidentified product with a weak maximum in the region of 300 mμ.

Even less can be said about the products of bromination of guanine. In the spectrum of the latter, the characteristic absorption band with a maximum at 252 mμ and an inflection at 266 mμ disappears (Fig. 3). However, absorption in the region 240–280 mμ is fairly large, and at 257 mμ the brominated base has a weakly expressed absorption maximum. On paper chromatography of brominated guanine, a series of weakly UV-absorbing spots is detected, corresponding to several reaction products.

Fig. 3. Spectrum of guanine. Designations 1-4 are the same as in Fig. 1

Figure 3: Fig. 3. Spectrum of guanine. Designations 1-4 are the same as in Fig. 1

Fig. 4. Rate of bromination of guanine (1), cytosine (2), uracil (3)

Figure 4: Fig. 4. Rate of bromination of guanine (1), cytosine (2), uracil (3)

Thus, bromination of uracil leads to the formation of 5,5-dibromo-4-oxyhydrouracil, saturated at the 4,5 bond. The spectrum of this compound is very close to the absorption spectrum of 4,5-dihydrobromouracil [12,13], the product of photolysis of uracil—4-oxyhydrouracil [14]—and its methylated derivatives [13,15]. With a considerable degree of probability it may be assumed that, on bromination of cytosine, a derivative saturated at the 4,5 bond is also formed.

Bromination of the four nitrogenous bases proceeds at different rates: uracil reacts almost instantaneously with bromine water $1:20$; adenine is not brominated at all in an acidic medium; guanine and cytosine occupy an intermediate position. The kinetics of bromination is shown in the graph (Fig. 4). The course of bromination depends primarily on the density

of the electron cloud at C_5. Attack at C_5 proceeds the more readily, the greater the negative charge of this carbon atom.

The addition reaction (which is what bromination of nitrogen bases is) is characteristic of unsaturated compounds of the aliphatic series and is not typical of aromatic derivatives. The heterocycles of purines and pyrimidines possess mixed properties, and their aromaticity depends primarily on the nature of the substituents. The oxo group at C_6 increases the unsaturation of the 4,5 bond of the ring, increases by the inductive route the negative charge at C_4, and thereby promotes bromination. On the other hand, the low electronegativity of N in the amino group at C_6 ultimately creates a reduced density of the electron cloud at C_5, and in this connection bromination should proceed at a slower rate. What has been said applies to cytosine and adenine, which are indeed brominated with more difficulty than uracil and guanine.

Fig. 3. Spectrum of guanine. Designations 1—4 are the same as in Fig. 1

Fig. 4. Rate of bromination of guanine (1), cytosine (2), uracil (3)

These purely qualitative judgments can be given a quantitative basis by using Pullman's calculated data [1,6]. Table 1 shows the values of the effective charges at C_5. Uracil has the greatest negative charge, adenine the smallest; cytosine and guanine occupy an intermediate position. The values of this parameter correspond to the experimentally observed course of bromination of nitrogen bases and confirm that an increase in the negative charge at C_5 favors addition of Br to this carbon. The magnitudes of the positive charges at C_4 deviate

somewhat from the order of distribution of the negative charges, but the C_4 charge of uracil is nevertheless greater than the C_4 charge of adenine.

The bond order $C_4 = C_5$, characterizing the degree of its aromaticity (or, conversely, unsaturation), deserves attention. The 4,5 bond of uracil is the most unsaturated, and adenine is the most aromatic. In the same order are arranged the values of free valence (rows 4 and 5), which characterize the reactivity of the C_4 and C_5 atoms. Thus, the calculated parameters of the electronic structure of nitrogen bases, obtained by the molecular-orbital method, agree with the behavior of these compounds during bromination.

The nature of the chemical transformations occurring during bromination predetermines the character of the change in the absorption spectrum. Addition of Br to pyrimidine and purine heterocycles occurs at the expense of π-electrons, and its prerequisite is incomplete delocalization of the π-electrons of the conjugated cyclic structure.

Table 1

Charges, bond orders, and free valences of C_4 and C_5 of pyrimidine and purine bases

Parameter	Uracil (lactam)	Cytosine (lactam-amine)	Guanine (lactam-amine)	Adenine (amine)
Charge C_5	−0.219	−0.169	−0.176	−0.087
Charge C_4	+0.146	+0.165	+0.023	+0.037
Bond order $C_4 = C_5$	0.819	0.758	0.630	0.576
Free valence C_5	0.526	0.449	0.222	0.159
Free valence C_4	0.434	0.445	0.140	0.138

The double 4,5-bond plays an important role in the formation of the conjugated system of nitrogenous compounds and in the appearance of intense absorption in the region 220–300 mμ. Indeed, uracil in solution exists predominantly in the lactam-lactam form, and its conjugated system is formed by the oxy group at C_6 and the 4,5-bond. During bromination this system is disrupted, and the characteristic absorption disappears. In cytosine in the lactam-amine form, the conjugated system is formed by three chromophoric groups (the oxy group at C_2, N_1, the C_6-bond, and the 4,5-bond). During bromination the system likewise loses its essential 4,5-bond component. The same relationships exist in the bromination of guanine.

During bromination of uracil, cytosine, and guanine, bromo derivatives are formed that are saturated at the 4,5-bond and have lost the characteristic absorption of the original base in the region 220–300 mμ.

The differences in the course of bromination of these bases are associated with the unequal degree of aromaticity of their rings and depend to a significant extent on the nature of the substituent at C_6 and its influence on the 4,5-bond. A decrease in the aromaticity of the 4,5-bond and an increase in the negative charge at C_5 favor bromination. The parameters of atoms C_5 and C_4, calculated by the molecular-orbital method, correspond to the empirically observed regularities in the bromination of nitrogenous bases. The changes in the absorption spectrum of nitrogenous bases in the region 220–300 mμ that occur during bromination depend on disruption of their conjugated chromophore system, caused by saturation of the 4,5-bond of the heterocycle.

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Note: Figure translations are in progress. See original paper for figures.