HALOGENATION OF THE COMPONENTS OF NUCLEIC ACIDS

# I. BROMINE DERIVATIVES OF THE COMPONENTS OF RIBONUCLEIC ACID

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The bromination of nucleic acids, nucleosides, and nucleotides of different degrees of phosphorylation has been studied intensively [1-3] and is used for the modification of nitrogenous bases. In addition to this, bromine derivatives of the components of nucleic acids are widely used for the introduction of a tritium label by reductive dehalogenation [4-9].

In the majority of publications, the bromination of protected derivatives is discussed; for example, methods have been described [1-3] for the bromination of unprotected AMP, UMP, and CMP. We have developed preparative methods for the synthesis and ion-exchange purification of 8-Br-AMP, 5-Br-UMP, and 5-Br-CMP, and also of 8-Br-GMP and 8-bromoguanosine. For the chromatographically pure bromine derivatives we have determined $R_f$ in various solvent systems on paper and in a thin layer of silica gel, and UV absorption spectra and molar extinction coefficients. There is almost no information on these properties in the literature.

## EXPERIMENTAL

8-Bromoadenosine 5'-Monophosphate (8-Br-AMP). A solution was made of 2 g (5.5 mmole) of the free acid AMP·$H_2O$ in a mixture of 20 ml of 1 M acetate buffer, pH 4.0, and 5 ml of 2 NaOH (to convert the AMP into the disodium salt) and it was diluted with a further 200 ml of acetate buffer. Then 52 ml of 0.216 M bromine water was gradually added (with frequent shaking). The mixture was left at room temperature in the dark for 12 h, after which the excess of bromine was reduced by the dropwise addition (using starch-iodide paper to monitor the process) of a 2 M solution of sodium bisulfite. To isolate the nucleotides we added an excess of 2 M barium acetate, filtered off the barium sulfate, and precipitated the barium salts with a fourfold volume of ethanol. The yield of 8-Br-AMP was 60% of theoretical.

8 Bromoguanosine 5'-Monophosphate (8-Br-GMP). The disodium salt of GMP 0.25 g (0.54 mmole) was dissolved in 10 ml of water, and 21 ml of 1 M acetate buffer, pH 3.0 and 2.95 ml of saturated bromine water were added. The reaction mixture was left at room temperature in the dark for 25 min, and then the reaction was stopped by the addition of 4 ml of 0.1 N sodium bisulfite. The nucleotides were extracted from the reaction mixture by adsorption on "Norit" activated carbon and were desorbed from the carbon with a 1% solution of ammonia in 50% ethanol. The yield of 8-Br-GMP was 65%.

5-Bromouridine 5'-Monophosphate (5-Br-UMP) and 5-Bromocytidine 5'-Monophosphate (5-Br-CMP). The bromination of the pyrimidine ribonucleoside 5'-monophosphates was performed in an acidic aqueous organic medium. To a weighed amount of the nucleotide (free acid!) was added a 0.5 N solution of $HNO_3$ and dioxane. After the formation of a homogeneous solution, a 1.5 N solution of bromine in $CCl_4$ (50% excess) was added rapidly. The reaction was performed at room temperature in the dark with vigorous stirring in order to prevent the separation of the emulsion into layers. Then the solvents and the excess of bromine were distilled off in vacuum from a rotary evaporator (at a bath temperature of 50-52°C). The syrupy residue was dissolved in 50 ml of 96% ethanol, and then the ethanol was distilled off completely. This operation was repeated another 3-4 times, the bath temperature being main-

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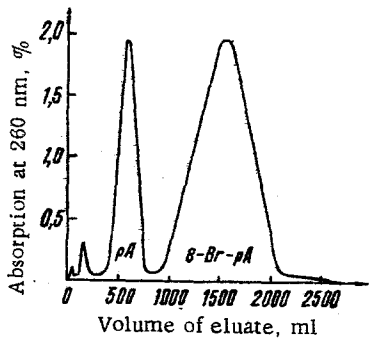


Fig. 1. Elution curve in the separation of the products of the bromination of AMP.

TABLE 1

| Compound | Washing with water, ml | Eluent I | | Eluent II | |
|---|---|---|---|---|---|
| | | composition | vol., ml | composition | vol. ml |
| 8-Br-AMP, Na salt<br>8-Br-GMP, Na salt | ~200<br>~250 | 0,2 N HCOOH<br>0,8 N $HCOONH_4$ + +0,4 N HCOOH | 650<br>~500 | 0,2 N HCOOH<br>0,8 N $HCOONH_4$ + +0,4 N HCOOH | 1000<br>~500 |
| 5-Br-UMP, Ba salt<br>5-Br-CMP, Ba salt<br>8-Br-guanosine | ~200<br>~200<br>~150 | 2 N HCOOH<br>0,1 N HCOOH<br>0.2 N HCOOH | 500<br>150<br>200 | 4 N HCOOH<br>0,5 N HCOOH<br>2 N HCOOH | 1000<br>150<br>250 |

TABLE 2

| Compound | Medium | $\lambda_{max}$, nm | $\lambda_{min}$, nm | $\varepsilon_{max} \times 10^{-3}$ | $\varepsilon_{260} \times 10^{-3}$ | $\frac{\varepsilon_{250}}{\varepsilon_{260}}$ | $\frac{\varepsilon_{280}}{\varepsilon_{260}}$ | $\frac{\varepsilon_{290}}{\varepsilon_{260}}$ |
|---|---|---|---|---|---|---|---|---|
| 8-Bromo-AMP, Ba salt | 0,01 N HCl | 262,5 | 230 | 17,4 | 16,8 | 0,69 | 0,49 | 0,15 |
| | $H_2O$ | 265 | 230 | 16,6 | 15,5 | 0,68 | 0,53 | 0,17 |
| | 0,01 N NaOH | 265 | 235 | 16,6 | 15,5 | 0,67 | 0,53 | 0,12 |
| 8-Bromo-GMP, Ba salt | 0,01 N HCl | 262 | 226 | 15,4 | 15,3 | 0,81 | 0,72 | 0,46 |
| | $H_2O$ | 262 | 224 | 15,2 | 15,0 | 0,81 | 0,73 | 0,47 |
| | 0,01 N NaOH | 272 | 235 | 13,5 | 12,2 | 0,74 | 1,04 | 0,63 |
| 5-Bromo-UMP, Ba salt | 0,01 N HCl | 280 | 243 | 8,1 | 4,3 | 0,58 | 1,88 | 1,47 |
| | $H_2O$ | 279 | 245 | 7,9 | 4,6 | 0,59 | 1,65 | 1,27 |
| | 0,01 N NaOH | 278 | 251 | 5,8 | 3,9 | 0,87 | 1,43 | 0,90 |
| 6-Bromo-CMP, Ba salt | 0,01 N HCl | 300 | 255 | 10,2 | 2,0 | 1,13 | 2,82 | 4,10 |
| | $H_2O$ | 290 | 262 | 7,5 | 4,2 | 1,25 | 1,59 | 1,81 |
| | 0,01 N NaOH | 290 | 262 | 7,6 | 4,1 | 1,23 | 1,47 | 1,75 |
| 8-Bromoguanosine | 0,01 N HCl | 260 | 225 | 16,9 | 16,9 | 0,83 | 0,66 | 0,40 |
| | $H_2O$ | 261 | 225 | 16,7 | 16,5 | 0,85 | 0,67 | 0,40 |
| | 0,01 N NaOH | 270 | 230 | 14,1 | 13,4 | 0,78 | 0,83 | 0,40 |

tained between 50 and 55°C. The conditions for the synthesis of the 5-Br-UMP and the 5-Br-CMP are given below:

| Initial nucleotide | Initial substance, mmole | 0.5 N $HNO_3$, ml | Dioxane, ml | Excess of Br, % | Time of the reaction, h | Yield of bromine derivative, % |
|---|---|---|---|---|---|---|
| CMP, free acid | 2.58 | 5.16 | 20.6 | 50 | 1.25 | 59 |
| UMP, free acid | 4.63 | 9.26 | 37.0 | 50 | 1.00 | 88 |

After the completion of the distillation with ethanol, the residue was dissolved in a small amount of water, the solution was accurately neutralized with a 1 N solution of NaOH,

and the nucleotides were precipitated in the form of the barium salts with a 2 N solution of barium acetate and a fourfold volume of ethanol.

8-Bromoguanosine. To 14.0 g (50 mmole) of guanosine was rapidly added 500 ml of freshly prepared 3% bromine water, and the flask was closed with a ground-in stopper and was shaken vigorously for 1-2 min. During this time, the guanosine was shaken for another 35 min at room temperature in the dark, and after this the precipitate was rapidly filtered off on a porous glass filter and was carefully washed with ~75 ml of cold water. Then the 8-bromoguanosine was crystallized three times from hot water. After the third crystallization, the 8-bromoguanosine consisted of a completely colorless microcrystalline powder. Yield ~58%.

Some Physicochemical Properties of the Bromine Derivatives. Ion-Exchange Chromatography. To purify the bromine derivatives obtained we used ion-exchange column chromatography on Dowex 1 × 8 resin in the formate form with stepwise elution with formic acid and mixtures of it with ammonium formate of various concentrations. We used a column with the dimensions 23 × 1.6 cm. After the introduction of the reaction product, the column was washed with water; appropriate eluents first eluted the unchanged nucleotides and then their bromine derivatives. Information on ion-exchange chromatography is given in Table 1, and Fig. 1 shows the elution curve in the separation of the products of the bromination of AMP.

Thin-Layer Chromatography. Standard "Silufol $UV_{254}$" plates were used with the following solvent systems: 1) butanol—acetic acid—water (5:2:3); 2) ethanol—1 M ammonium acetate, pH 7.5 (5:2); and 3) isopropanol—ammonia (concentrated)—water (7:1:2). The average $R_f$ values of the bromine derivatives of the RNA components that were synthesized are given below:

| Compound | System 1 | System 2 | System 3 |
|---|---|---|---|
| 8-Bromo-AMP, Ba salt | 0.34 | 0.22 | 0.26 |
| 8-Bromo-GMP, Ba salt | 0.26 | 0.14 | 0.19 |
| 5-Bromo-UMP, Ba salt | 0.39 | 0.24 | 0.13 |
| 5-Bromo-CMP, Ba salt | 0.27 | 0.16 | 0.14 |
| 8-Bromoguanosine | 0.65 | 0.85 | 0.70 |

Paper chromatography was performed by the ascending method at room temperature on Whatman No. 1 paper. The following solvent systems were used: 1) butanol—acetic acid—water (4:1:5); 2) butanol—propanol—ammonia (concentrated)—water (7:5:7:2); and 3) ethanol—1 M ammonium acetate, pH 7.5 (5:2). The mean $R_f$ values of the bromine derivatives of the RNA components that were synthesized are given below:

| Compound | System 1 | System 2 | System 3 |
|---|---|---|---|
| 8-Bromo-AMP, Ba salt | 0.38 | 0.24 | 0.18 |
| 8-Bromo-GMP, Ba salt | 0.34 | 0.16 | 0.21 |
| 5-Bromo-UMP, Ba salt | 0.36 | 0.11 | 0.23 |
| 5-Bromo-CMP, Ba salt | 0.31 | 0.19 | 0.24 |
| 8-Bromoguanosine | 0.47 | 0.39 | 0.80 |

Spectral Characteristics. The UV spectra were taken on a Perkin—Elmer model 402 spectrometer. The UV spectral characteristics of the bromine derivatives are given in Table 2.

## SUMMARY

Preparative methods have been worked out for the bromination of unsubstituted AMP, GMP, UMP, CMP, and guanosine, and also methods for the isolation and purification of the bromine derivatives.

Some physicochemical characteristics (UV spectra, $R_f$ values on thin-layer and paper chromatography, coefficients of millimolar extinction) of the bromine derivatives of the components of RNA obtained have been determined.

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# HALOGENATION OF THE COMPONENTS OF NUCLEIC ACIDS

## II. BROMINE DERIVATIVES OF DEOXYRIBONUCLEOSIDE 5'-MONOPHOSPHATES

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The present work is a continuation of investigations on the synthesis of halogen derivatives of components of nucleic acids [1]. We have synthesized, purified, and characterized bromine derivatives of a number of deoxyribonucleoside 5'-monophosphates.

For the components of DNA, just as for RNA, there are a number of methods of halogenation which can be divided into two groups — halogenation in nonaqueous media [2, 3] and in aqueous media [2, 4]; protected derivatives are generally halogenated. In the pyrimidine series, the conditions of halogenation of ribo and deoxyribo derivatives are similar and sometimes practically identical. The halogenation of the purine deoxyribo derivatives has been considered in various publications [5-8], which relate mainly to deoxynucleosides. A method for the halogenation of deoxyadenosine 3',5'-cyclophosphate is described in a German patent [9] in which the 3',5'-cyclophosphate grouping protects both hydroxyls of the deoxyribose residue.

## EXPERIMENTAL

8-Bromodeoxyadenosine 5'-Monophosphate (8-Br-dAMP). A mixture of 1.0 g (3.0 mmole) of dAMP (free acid), 10 ml of 1 M acetate buffer with pH 4.0, and 3 ml of 2 N NaOH (to convert the nucleotide into the disodium salt) was stirred until the solid had dissolved, and then another 100 ml of buffer was added. After this, 34 ml of saturated bromine water (about 2.25 mmole of bromine to 1 mmole of dAMP) was gradually added. The mixture was left in the dark at room temperature for 12 h.

The excess of bromine was decomposed by the addition of 2 M $NaHSO_3$ (~5 ml). The $SO_4$ ions formed were precipitated with ~5-7 ml of a 2 M solution of barium acetate. The precipitate of $BaSO_4$ was filtered off, and the filtrate was treated with 5-6 ml of 2 M barium acetate (~100% excess of $Ba^{2+}$), and a fourfold volume of ethanol was gradually added. After the ripening of the precipitate for 12 h in the refrigerator, the supernatant liquid was separated off by decantation. The residual suspension was centrifuged (6000 rpm, 20 min). The precipitate of barium salt was carefully washed three times in the centrifuge with a mixture of ethanol and water (4:1 by volume) and was dried in vacuum over $P_2O_5$. The barium salt of 8-Br-dAMP was obtained with a yield of 75-80% and did not require purification (the residual amount of dAMP and other impurities determined by analytical ion-exchange chromatography amounted to ~1%).

8-Bromodeoxyguanosine 5'-Monophosphate (8-Br-dGMP). A mixture of 0.29 g (0.74 mmole) of the disodium salt of dGMP, 29 ml of 1 M acetate buffer, pH 3.0, and 4.5 ml of saturated bromine water (30% excess of bromine) was kept in the dark at room temperature for 25 min. The reaction was stopped by the addition of 2 ml of a 0.1 N solution of $NaHSO_3$, and the

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