6. V. M. Vdovenko et al., Radiokhimiya, 14, 457 (1972).
7. G. G. Gusev et al., in: The Production of Isotopes [in Russian], Moscow (1973), p. 165.
8. I. F. Tupitsyn et al., in: The Production of Isotopes [in Russian], Moscow (1973), p. 158.
9. N. S. Marchenkov, Author's Abstract of Candidate's Dissertation, Moscow (1974).

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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TABLE 1

Compound	Medium	λ_{max}, nm	λ_{min}, nm	$E_{max} \times 10^{-3}$	$E_{260} \times 10^{-3}$	$\frac{E_{250}}{E_{260}}$	$\frac{E_{280}}{E_{260}}$	$\frac{E_{290}}{E_{260}}$
8-Bromodeoxy-AMP (barium salt)	0,01 N HCl	263	230	13,20		0,67	0,51	0,14
	H_2O	265	232	12,30		0,65	0,55	0,14
	0,01 N NaOH	265	232	12,60		0,65	0,55	0,14
8-Bromodeoxy-GMP (barium salt)	0,01 N HCl	260	—	14,54	14,54	0,83	0,73	0,44
	H_2O	260	225	15,26	15,26	0,82	0,72	0,45
	0,01 N NaOH	272	234	13,58	12,18	0,75	1,00	0,58
5-Bromodeoxy-CMP (barium salt)	0,01 N HCl	298	254	6,37	1,21	0,97	3,22	4,65
	H_2O	288	260	4,86	2,51	1,21	1,74	1,86
	0,01 N NaOH	288	260	4,86	2,51	1,23	1,74	1,86

nucleotides were isolated by adsorption on activated carbon. The ammonium salts of the nucleotides were desorbed and were separated by ion-exchange chromatography as described previously for the case of 8-Br-GMP [1]. After concentration by means of activated carbon, 8-Br-dGMP was obtained with a yield of ~80%.

5-Bromodeoxycytidine 5'-Monophosphate (5-Br-dCMP). A mixture of 0.2 g (0.65 mmole) of dCMP (free acid!), 1.3 ml of 0.5 N HNO_3, 5.2 ml of dioxane, and 1.3 ml of a 1.5 N solution of bromine in CCl_4 was stirred vigorously in the dark at room temperature for 75 min. Then the solvent and the excess of bromine were distilled off in vacuum from a rotary evaporator at a bath temperature of 37°C. The residue in the flask was dissolved in 15 ml of ethanol, and the solution was again evaporated to dryness. This operation was repeated seven times. The residue was dissolved in 6 ml of water and the solution was brought to a pH of 6.0-6.5 with a 0.2 N solution of NaOH (~5 ml), after which an excess of 2 M barium acetate was added and the barium salts were precipitated with a fourfold volume of ethanol. The precipitate was separated off by centrifuging and was washed three times with a mixture of ethanol and water (4:1 by volume). The barium salts of the nucleotides were dissolved in water and subjected to ion-exchange chromatographic separation as described previously for the case of 5-Br-CMP [1]. The 5-Br-dCMP was isolated from the formic acid eluent by means of "Norit" activated carbon. After desorption, the ammonium salt was converted into the barium salt by means of barium acetate and ethanol, and this was dried in vacuum over P_2O_5. The 5-Br-dCMP was obtained with a yield of ~70%.

Some Physicochemical Properties of the Bromine Derivatives. Thin-layer chromatography. For thin-layer chromatography we used standard "Silufol UV_{254}" plates. We used the same solvent systems as in the preceding paper [1]. The mean R_f values of the bromine derivatives of DNA that were synthesized are as follows:

Compound	System 1	System 2	System 3
8-Bromodeoxy-AMP	0.32	0.33	0.39
8-Bromodeoxy-GMP	0.39	0.31	0.20
5-Bromodeoxy-CMP	0.27	0.24	0.15

Paper chromatography. Chromatography was performed by the ascending method at room temperature on Whatman No. 1 paper. The solvent systems given in the preceding paper [1] were used. We give the mean R_f values of the bromine derivatives of the components of DNA that were synthesized:

Compound	System 1	System 2	System 3
8-Bromodeoxy-AMP	0.23	0.30	0.52
8-Bromodeoxy-GMP	0.20	0.31	0.46
5-Bromodeoxy-CMP	0.21	0.24	0.43

Spectral characteristics. The UV spectra were taken on a Perkin-Elmer model 402 spectrophotometer. Some spectral characteristics are given in Table 1.

SUMMARY

Preparative methods have been worked out for the bromination of unprotected deoxy-AMP, deoxy-GMP, and deoxy-CMP and methods have been devised 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 DNA components obtained have been determined.

LITERATURE CITED

1. K. S. Mikhailov et al., Khim. Prirodn. Soedin., 522 (1976) [in this issue].
2. N. K. Kochetkov and É. I. Budovskii (editors), The Organic Chemistry of the Nucleic Russian] (1970), p. 312.
3. J. Smrt and F. Šorm, Collect. Czech. Chem. Commun., 25, 553 (1960).
4. P. K. Chang and A. D. Welch, J. Med. Chem., 6, 428 (1963).
5. R. E. Holmes and R. K. Robins, J. Am. Chem. Soc., 86, 1242 (1964).
6. D. Nowotny and D. Lipkin, Monatsh., 96, 125 (1965).
7. D. Lipkin et al., J. Biol. Chem., 238, 2249 (1963).
8. R. A. Long, R. K. Robins, and L. B. Townsend, J. Org. Chem., 32, 2751 (1967).
9. G. Wiemann et al., German Federal Republic Patent No. 1922172 (Class CO7d, 61 k).

ACTIVATION OF TRYPSINOGEN BY AN ACID PROTEINASE FROM *Aspergillus awamori*

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The activation of trypsinogen can take place autocatalytically — under the action of trypsin — and with the participation of a number of other proteolytic enzymes [1], especially those of microbiological origin [1, 2]. We have investigated the possibility of the activation of the trypsin precursor by acid proteinases — pepsin, rennin, and the enzyme produced by the mold fungus *Aspergillus awamori*.

Pepsin did not activate tripsinogen either at pH 3.0 or at pH 5.0, which is in harmony with the results of other authors [2]. In our experiments, rennin also showed no capacity for activating trypsinogen. A high activating capacity was shown only by the proteinase from *A. awamori*. We have found that the optimum pH for the activation of trypsinogen by this enzyme is 3.0 (0.057 M CH_3COOH). This excludes the autoactivation of trypsinogen, which takes place readily at higher pH values in the presence of even traces of trypsin [3]. On the other hand, at pH 3.0 the proteinase from *A. awamori* possesses a fairly high proteolytic activity, ensuring a high rate of activation and, moreover, trypsin is most stable at this pH value.

In contrast to the autoactivation of trypsinogen, the presence of Ca^{2+} ions is not essential for the activation of the zymogen by the proteinase from *A. awamori*, since we obtained similar results when using 0.05 M $CaCl_2$ and in the absence of Ca^{2+}.

At a ratio of trypsinogen to the *A. awamori* proteinase of 1000:1 (Fig. 1a), activation is practically complete in 1 h. The enzyme from *A. awamori*, in addition to bringing about the specific cleavage of trypsinogen with the formation of trypsin, also performs the nonspecific proteolysis of the protein, which leads to a loss of trypic activity. Thus, at a ratio

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