a hemoglobin activity of 1.0 activity unit/optical unit in 12 ml of 0.1 M acetate buffer, pH 5.6. Chromatography on the Sepharose 4B—DNP-ethylenediamine sorbent was carried out under the same conditions.

SUMMARY

1. The chromatography of the carboxylic proteinases porcine pepsin, aspergillopepsin A, and chymosin on the hydrophobic sorbent Sepharose 4B—DNP-hexamethylenediamine has been studied. It has been shown that the nature of the binding of the proteinases with the sorbent depends on the pH.

2. A shortening of the length of the carbohydrate chain of the ligand by four methylene units substantially weakens the interaction of pepsin with the sorbent.

3. With chymotrypsin and pepsin as examples, the possibility has been shown of using ionic effects for separating these enzymes.

LITERATURE CITED

1. V. M. Stepanov, G. I. Lavrenova, V. P. Borovikova, and C. N. Balandina, J. Chromatogr., 104, 373 (1975).
2. M. M. Chernaya, K. Adli, G. I. Lavrenova, and V. M. Stepanov, Biokhimiya, 41, 732 (1976).
3. R. Jost, T. Miron, and M. Wilchek, Biochem. Biophys. Acta, 362, 75 (1974).
4. G. I. Boellgast and W. H. Fishman, Biochem. J., 141, 103 (1974).
5. G. S. Doley, M. J. Harvey, and P. D. G. Dean, FEBS Lett., 65, 87 (1976).
6. G. G. Kovaleva, M. P. Yusunova, E. N. Lysogorskaya, G. N. Balandian, and V. M. Stapanov, Biokhimiya, 42, 534 (1977).
7. V. M. Stepanov, G. I. Levrenova, K. Adli, M. V. Gonchar, G. N. Balandina, M. M. Slavinskaya, and A. Ya. Strongin, Biokhimiya, 41, 294 (1976).
8. V. M. Stepanov and T. I. Greil', Biokhimiya, 28, 540 (1963).
9. M. L. Anson, J. Gen. Physiol., 22, 79 (1938).
10. L. P. Alekseenko, in: Modern Methods in Biochemistry, V. N. Orekhovich (ed.), [in Russian], Moscow (1968), p. 120.
11. J. Porat, K. Aspberg, H. Drevin, and R. Axen, J. Chromatogr. 86, 53 (1973).

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

I. CIRCULAR DICHROISM SPECTRA OF 8-BROMINE-SUBSTITUTED PURINE NUCLEOTIDES

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A number of communications [1-4] have been devoted to the study of the optical activity of natural derivatives of purine and pyrimidine compounds. The information given in them permits an explanation of the features of the electronic structures of the compounds investigated and, furthermore, provides the possibility of quantitatively connecting a change in optical properties with a change in conformational states [5-7]. The introduction of a halogen atom into the heterocyclic base of a purine or a pyrimidine nucleotide changes the energy of the electronic transitions and must also change the conformation of the compound as compared with the initial, unhalogenated, state. This change is connected mainly with the angle of rotation of the heterocyclic base around the glycosidic bond (φ_{CN}). According to Donohue and Trueblood [8], the compound has the anti conformation when $\varphi_{CN} = -30\pm45°$ and the syn conformation when $\varphi_{CN} = 150\pm45°$.

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This has been shown in publications [9-16] for the compounds of interest to us in the crystalline state (mainly nucleosides). In an investigation of the optical activity of halogenated nucleosides it has been found that the introduction of a halogen substituent changes the profiles of the optical rotary dispersion (ORD) and circular dichroism (CD) curves in comparison with the profiles of the curves of the initial compound. The authors concerned connected this with a change in φ_{CN} for the compounds investigated. Taking such changes into account in, for example, CD spectra enables us to compare the structural changes of halogen-substituted fragments of nucleic acids with features of their behavior in chemical and biochemical transformations. Here we must mention the extremely small extent to which the CD spectra of bromine-substituted derivatives of purine and, particularly, pyrimidine nucleoside 5'-monophosphates, both of the ribo and of the 2'-deoxyribo series, have been studied.

In the present paper we describe the results of a study of the CD spectra of 8-bromine-substituted purine nucleotides. As the objects for investigation we selected the diammonium salts of 8-bromoadenosine 5'-monophosphate (8-bromo-AMP), of 8-bromoguanosine 5'-monophosphate (8-bromo-GMP), and of 8-bromo-2'-deoxyguanosine 5'-monophosphate (8-bromo-dGMP), and the barium salt of 8-bromo-2'-deoxyadenosine 5'-monophosphate (8-bromo-dAMP).

The CD spectra of 8-bromo-AMP and of 8-bromo-dAMP are shown in Fig. 1 and those of 8-bromo GMP and 8-bromo dGMP in Fig. 2. The spectral parameters of the compounds investigated are given in Table 1.

In the 200-300 nm region three Cotton effects (CEs) were recorded which corresponds to $\pi-\pi^*$ transitions in the B_{2u}, B_{1u}, and E_{1ua} bands (localized, respectively, in the 280-265, 250-240, and 220-200 nm regions). The CD spectra of the compounds investigated had changed in comparison with the corresponding spectra of the unbrominated analogs given in the literature. Thus, all the CD curves of the nucleotides investigated had a well-defined positive CE in the B_{2u} spectral region and a high amplitude of the E_{1ua} band. As can be seen from Fig. 1, the CD spectra of 8-bromo-AMP and 8-bromo-dAMP show a small negative B_{1u} CE. All the bands of 8-bromo-GMP and 8-bromo-dGMP are positive, and the B_{1u} band is probably masked by the stronger E_{1ua} band and is not recorded (Fig. 2). It has been shown that a voluminous substituent at C_8 excludes the possibility of the formation of the anti-conformation of purines in solution [7]. On considering the results of the analysis of the optical activity of the purines [17, 18], also, it may be concluded that a positive CE in the B_{2u} region is due to the syn conformation of the compounds investigated over a wide pH range and in methanol. The small changes in the amplitudes of the B_{2u} band are probably determined by conformational rearrangements of the molecule. The latter are connected with a change in the angle of rotation φ_{CN} or of the dihedral angle between the plane of the heterocyclic base and the ribose, or with both these factors. In actual fact, starting from general ideas developed, e.g., by Tinoco [19] it follows that the magnitude of the rotational force of a nucleoside depends on the angle between the directions of the corresponding electronic transitions in the ribose and the base, i.e., both on the angle φ_{CN} and on the dihedral angle.

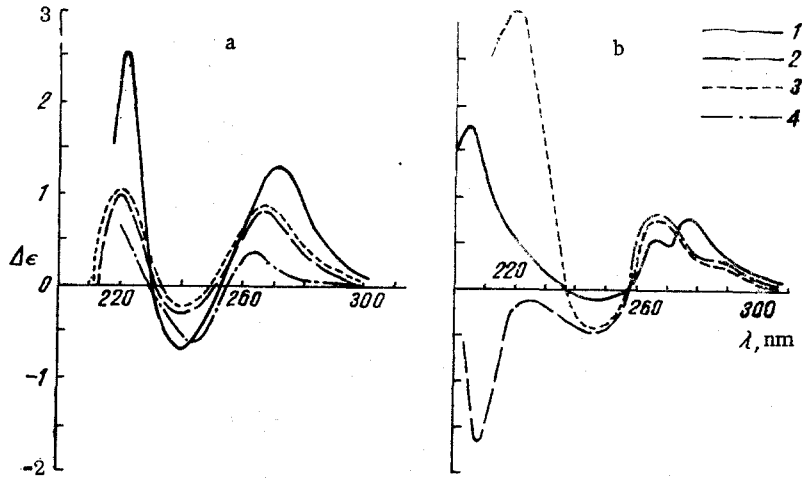

Fig. 1. CD spectra of 8-bromo-AMP (a) and of 8-bromo-dAMP (b): 1) pH 2; 2) pH 7; 3) pH 12; 4) methanol.

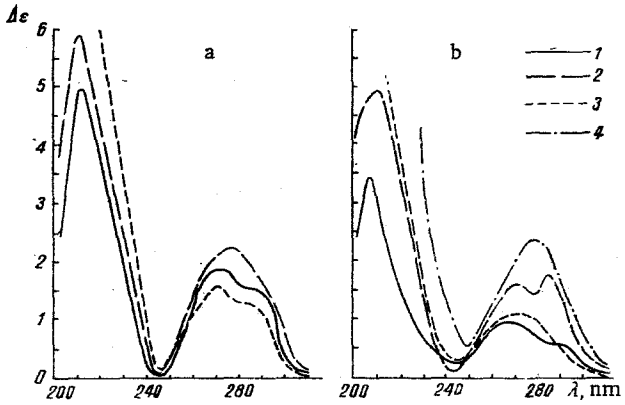

Fig. 2. CD spectra of 8-bromo-GMP (a) and 8-bromo-dGMP (b). Arbitrary symbols the same as in Fig. 1.

In the CD spectra of 8-bromo-AMP (Fig. 1a) in an acid medium the amplitude of the B_{2u} band rises, but at pH 7 and above it ceases to change. In view of the low rotational freedom of the base around the N-glycosidic bond because of the presence of the voluminous substituent in the 8-bromo-AMP molecule, this increase in the $\Delta\varepsilon$ value of the B_{2u} band may be connected with a conformational rearrangement, namely with a decrease in the dihedral angle or with the so-called "folding" of the molecule. Such "folding" of the molecule is explained by the existence of an intramolecular hydrogen bond between the N_3 atom of the adenine nucleus and the 2'-hydroxy group of the ribose. In neutral and alkaline media, such a bond is probably weakened. In methanol (Fig. 1a, curve 4), the amplitude of the B_{2u} band is a minimum and this is apparently connected with a maximum value of the dihedral angle under these conditions.

In the CD spectra of 8-bromo-dAMP (Fig. 1b) there is also a pH effect on the transition corresponding to the B_{2u} band. In an acid medium there is one more long-wave maximum (277 nm) on the CD curve. Its disappearance in neutral and alkaline media, and also the absence of appreciable changes in the UV spectrum of 8-bromo-dAMP in these media permit us to assign this maximum to an optically active n-π* transition. The nature and origin of this transition are apparently connected with the heteroatoms of the adenine nucleus and will not be considered in detail in the present communication. In contrast to the ribo derivative, the $\Delta\varepsilon$ value of the B_{2u} band is a minimum in an acid medium. This confirms the hypothesis of the nucleotide structure of the syn type through the formation of an intramolecular hydrogen bond with the heterocyclic base. In actual fact, in an acid medium a strengthening of the intramolecular hydrogen bond with the participation of the 2'-hydroxyl is observed which is accompanied by an increase in $\Delta\varepsilon$ of the B_{2u} band of the ribonucleotide. The absence of such a bond

TABLE 1. Spectral Characteristics of 8-Bromo-Substituted Purine Nucleotide

Compound	Medium	UV spectrum		CD spectrum		
		λ_{max}, nm ($\varepsilon_{max}\times10^{-3}$)	λ_{min}, nm ($\varepsilon_{min}\times10^{-3}$)	E_{1ua} λ(Δε)	B_{1u} λ (Δε)	B_{2u} λ (Δε)
8-Bromo-AMP	pH 2	264 (17,4)	230	222 (+2,50)	240 (—0,70)	272 (+1,30)
	pH 7	265 (16,6)	230	220 (+1,00)	242 (—0,30)	267 (+0,80)
	pH 12	265 (16,6)	235			
	Methanol	265 (16,6)	230	—	245 (—0,51)	265 (+0,38)
8-Bromo-deoxy-AMP	pH 2	264 (12,8)	230 (2,5)	205 (+1,75)	254 (—0,05)	265 (+0,51)
	pH 7	265 (12,3)	232 (3,1)	209 (—1,70)	245 (—0,35)	267 (+0,75)
	pH 12	264 (12,3)	232 (3,00)	221 (+3,00)	245 (—0,35)	267 (+0,75)
8-Bromo-GMP	pH 2	260 (15,0)	222 (2,8)	211 (+4,90)	—	272 (+1,80)
	pH 7	262 (15,2)	223 (3,9)	212 (+5,85)	—	278 (+2,15)
	pH 12	266 (13,6)	222 (2,8)	. . . . .	245 (+0,15)	272 (+1,60)
8-Bromo-deoxy-GMP	pH 2	255 (12,2)	228 (6,62)	207 (+3,45)	245 (+0,15)	268 (+0,90)
	pH 7	261 (15,2)	223 (2,70)	210 (+4,85)	—	272 (+1,55)
	pH 12	267 (13,7)	223 (0,74)	. . . . .	245 (+0,15)	272 (+1,10)
	Methanol	262 (15,3)	225 (2,8)	. . . . .	251 (0,40)	280 (2,25)

in a 2'-deoxyribo derivative leads to the opposite effect — the $\Delta\varepsilon$ value of the B_{2u} band is smaller in an acid medium (Table 1). The existence of an intramolecular hydrogen bond in a purine nucleotide having the syn conformation is also indicated by information on the change in the chemical shift in the NMR signal of the H_2' proton in brominated and unbrominated ribonucleotides [7]. In the present case, the difference in the pH dependences of the CD spectra of 8-bromine-substituted ribo- and deoxyribonucleotides gives additional evidence in favor of the existence of an intramolecular hydrogen bond with the participation of the 2'-hydroxyl of the ribose in nucleotides of the syn type.

In the CD spectra of 8-bromo-GMP (Fig. 2a) and 8-bromo-dGMP (Fig. 2b) the maximum value of the B_{2u} CE is observed in neutral aqueous solution. Just as in the case of the brominated adenine nucleotides investigated (Fig. 1), in 8-bromo-GMP the minimum value of the amplitude of the B_{2u} band is in an alkaline medium and for 8-bromo-dGMP it is in an acid medium. These facts also suggest the existence of an intramolecular hydrogen bond with the participation of the ribose 2'-hydroxy for reasons similar to those considered for the adenine nucleotides. However, in a less polar solvent — methanol — an increase in the amplitude of the B_{2u} band of the 8-bromo-GMP molecule takes place and this is probably accompanied by a still more pronounced "folding" of the molecule. The reason for this apparently lies in features of the electronic structure of the guanine nucleus.

Thus, small changes in the amplitude of the B_{2u} band probably reflect conformational changes of the molecule studied and, namely, a change in the dihedral angle between the plane of the heterocyclic base and that of the ribose residue. In these circumstances, some changes in the angle of rotation φ_{CN} are also possible, but these changes are limited by the low rotational freedom of the purine base around the N-glycosidic bond.

It can be seen from Figs. 1 and 2 and Table 1 that the CD spectra of the compounds investigated are characterized by a fairly large amplitude of the E_{1ua} band. As Ikehara et al. have reported [7], the 5'-phosphate group has an influence on the CD spectrum of 8-bromo-AMP and 8-bromo-GMP, particularly in the 200-220 nm region. The experimental results obtained confirm this (Figs. 1 and 2). Such changes in the CD spectra can be interpreted as a steric interaction of the chromophore of the base and of the negatively charged phosphate group. Such interaction appears most strongly, probably, in the case of guanine nucleotides having positively charged groups in the heterocyclic base (for example, an amino group at C_2). As a a result of the existence of such interaction, the number of most probable rotational stereomers falls considerably. This can apparently explain the fact that the CD curves of the 8-bromo-substituted guanine nucleotides are positive.

EXPERIMENTAL

The bromine-substituted purine nucleotides were synthesized, isolated, and characterized by published methods [20, 21]. The CD spectra were recorded on a Cary-60 spectropolarimeter with a 6001 attachment. The slit program was set at a spectra width of 15 Å over the whole range of recording (200-300 nm). The measurements were performed in a quartz cell 1.00 cm thick at a temperature of 27°C. The concentrations of the compounds investigated were 1.2-1.4 optical density units at the absorption maximum. The absorption spectra were recorded on a Unicam 800 spectrophotometer in a quartz cell 1 cm thick at room temperature.

SUMMARY

The CD spectra of 8-bromo-AMP, 8-bromo-dAMP, 8-bromo-GMP, and 8-bromo-dGMP in acid, neutral, and alkaline media and, in some cases, in methanol have been obtained. In the 200-300 nm region three Cotton effects have been recorded which correspond to $\pi-\pi^*$ in the B_{2u}, B_{1u}, and E_{1ua} bands (280-265, 240-250, and 200-220 nm, respectively). The CD spectra obtained differ from the spectra known in the literature for unbrominated compounds of this class. It has been shown that in ribonucleotides the syn conformation is stabilized by the formation of an intramolecular hydrogen bond with the participation of the ribose 2'-hydroxyl and the N_3 atom of the purine base. The 5'-phosphate group in the molecule of a purine nucloside of the syn type changes the CD profile, particularly in the short-wave region of the spectrum, as compared with the CD spectra of the unbrominated analogs given in the literature.

LITERATURE CITED

1. D. W. Miles, R. K. Robins, and H. Eyring, Proc. Nat. Acad. Sci. U.S.A., 57, 1138 (1967).

2. J. T. Yang, T. Samejima, and P. K. Sarkar, Biopolymers, 4, 623 (1966).
3. J. T. Yang and T. Samejima, Progr. Nucl. Acid Res. Mol. Biol., 9, 223 (1969).
4. D. W. Miles, M. J. Robins, R. K. Robins, and H. Eyring, Proc. Nat. Acad. Sci. U.S.A., 62, 22 (1969).
5. G. T. Rogers and T. L. V. Ulbricht, Biochem. Biophys. Res. Commun., 39, 419 (1970).
6. D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Insdeep, and H. Eyring, J. Am. Chem. Soc., 93, 1600 (1971).
7. M. Ikehara, S. Uesugi, and K. Yoshida, Biochemistry, 11, 830 (1972).
8. J. Donohue and K. N. Trueblood, J. Mol. Biol., 2, 373 (1962).
9. S. Fujii, T. Fujiwara, and K.-I. Tomita, Nucl. Acid Res., 3, 1985 (1976).
10. C. E. Bugg and V. Thewalt, Biochem. Biophys. Res. Commun., 37, 623 (1969).
11. A. E. Haschemeyer and H. M. Sobell, Acta Crystallogr., 19, 125 (1965).
12. A. E. Haschemeyer and A. Rich, J. Mol. Biol., 27, 369 (1967).
13. H. R. Wilson, Nature (London), 225, 545 (1970).
14. J. Iball, C. H. Morgan, and H. R. Wilson, Nature (London), 209, 1230 (1966).
15. G. H.-Y. Lin and M. Sundaralingam, J. Am. Chem. Soc., 94, 2572 (1972).*
16. P. A. Hart and J. P. Davis, J. Am. Chem. Soc., 94, 2572 (1972).
17. N. N. H. Teng, M. S. Itzkowitz, and I. Tinoco, J. Am. Chem. Soc., 93, 6257 (1971).
18. C. A. Bush, J. Am. Chem. Soc., 95, 214 (1973).
19. J. Tinoko, Advan. Chem. Phys., 4, 113 (1962).
20. K. S. Mikhailov, N. S. Marchenkov, V. L. Chichikina, V. A. Orlova, and N. F. Myasoedov, Khim. Prir. Soedin., 522 (1976).
21. N. S. Marchenkov, K. S. Mikhailov, V. A. Mikhailov, V. A. Orlova, and N. F. Myasoedov, Khim. Prir. Soedin., 525 (1976).

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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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