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SYNTHESIS AND SPECTRAL PROPERTIES OF SOME DINUCLEOTIDE ANALOGUES CONTAINING BROMINE IN 5 - POSITION OF PYRIMIDINE MOIETIES

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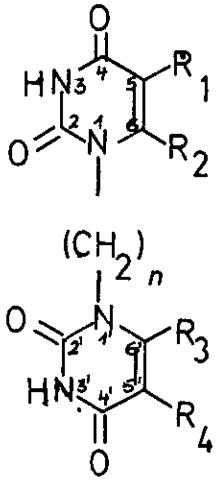
Abstract —— The bromination of pyrimidine bases in dinucleotide analogues has been studied. The structures of the brominated products have been established on the basis of analytical and spectral data.

1,1'-Polymethylenebis-(5-alkyl)uracils (I) introduced primarily by N.J. Leonard[1] as simplified models of dinucleotides, appeared very useful for studies of non-bonding interactions of bases and their intramolecular [2+2] photodimerization reactions[2,3]. In our former studies we investigated the influence of the size of alkyl substituents in 5 and 6 positions of the uracil moiety on the spectral and photochemical properties of model compounds. Consequently we focused our interest on the dinucleotide analogues halogenated in uracil part of the molecule.



I

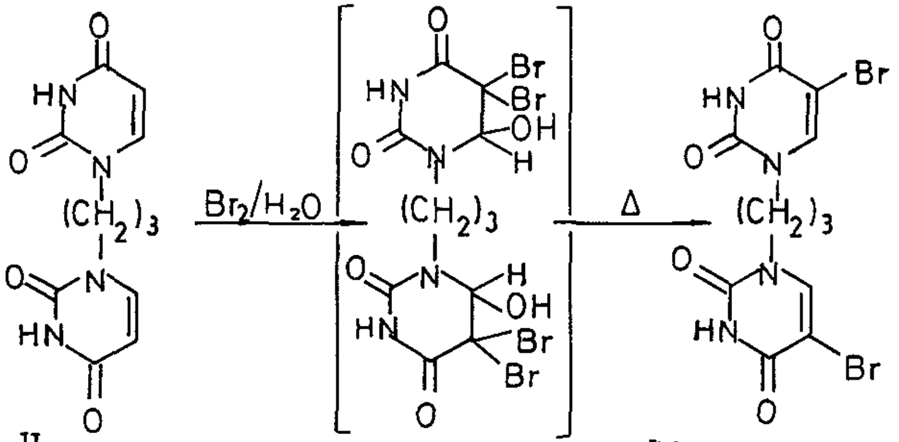
In this paper we present our preliminary results on synthesis and spectral properties of some new trimethylenebis-uracils having the bromine atom in the 5,5'-positions and/or only in the 5-position of I. It is known[4] that 5-bromouracil incorporated instead of thymine into DNA strain causes significant increase of its sensitivity upon interaction with ultraviolet radiation and it also gives other photoproducts than uracil and alkyl-uracils[5]. 5-Bromouracil as a chemical substance has mutagenic properties also[6], however, some of halopyrimidine nucleosides have potent medicinal properties. For example, 5-iododeoxyuridine[7], and 1-(tetrahydro-2-furanyl)-5-fluorouracil[8] have been used clinically as antiviral and antitumor agents, respectively.

RESULTS AND DISCUSSION.

Surprisingly well elaborated and effective methods for the synthesis of 1,1′-polymethylenebis- 5-alkyl uracils[1,9] completely failed in case of 5-bromouracil. Direct alkylation of 5-bromouracil with 1-(3-bromopropyl)-5-bromouracil yielded the product whose structure is now under elucidation. The application for the synthesis of bis-trimethylsilyl-derivative of 5-bromouracil yielded the desired compound only in 10% yield.

According to the literature data[10-13], many 5-bromouracil derivatives were synthesized by the direct bromination reaction. This method requires preparation of uracil skeleton with substituents in proper positions and introduction of bromine into the molecule takes place in the last step of synthesis which usually gives above 80% of yield.

We have adapted this approach for the synthesis of 1,1′-trimethylenebis-(5-bromo)-uracil (IV) which is shown in Scheme 1.

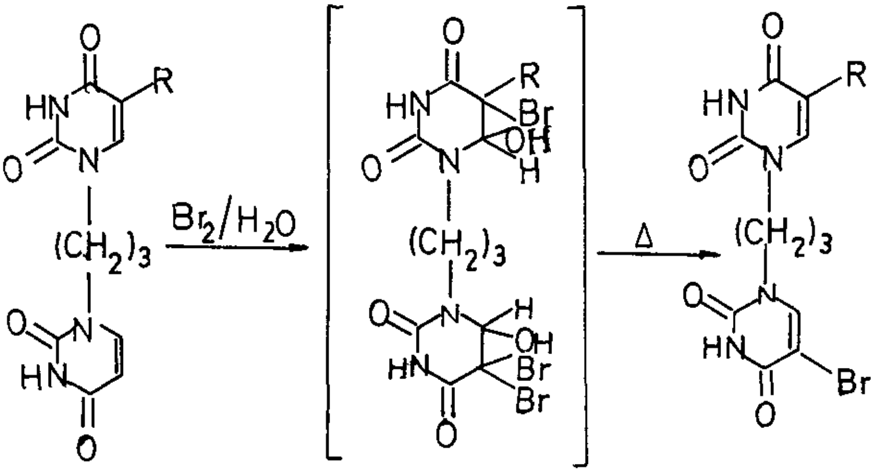


Scheme 1

This method uses 1,1′-trimethylenebis-uracil (II) as a substrate which is subjected to the bromination reaction under excess of bromine to give desired IV in 80% yield. We did not try to isolate the intermediate product III expected according to the mechanism of the bromination reaction of uracil and its derivatives[14-16]. This intermediate product was unstable in acidic medium and at elevated temperature was converted to IV, similarly as 5,5-dibromo-6-hydroxy-6-hydrouracil and its 1,3-dimethyl derivative which are transformed to 5-bromouracil and its 1,3-dimethyl derivative, respectively[14].

Because the last described method of synthesis of IV is superior to the former one, in the next step of this work we decided to adapt it to the synthesis of trimethylenebis-uracils possessing one bromine atom in the 5-position and an alkyl group in the 5′-position.

In the literature there are some different views concerning the properties of 5-bromo-6-hydroxy-adducts of thymine and derivatives. Moore et al.[15] have suggested the ring opening of thymine and thymidine HOBr addition products on heating in weakly acidic solution. On the other hand, Shugar et al[17] have shown, that the 5-bromo-6-hydroxy-adducts of thymine and thymidine at pH about 1 are transformed to the starting substances. This finding permitted us to extend the bromination reaction on trimethylenebis-(5-monoalkyl)-uracils for synthesis of trimethylenebis-(5-bromo, 5′-alkyl)-uracils (Scheme 2).



V, R= CH_3
VI, R= C_2H_5

VII

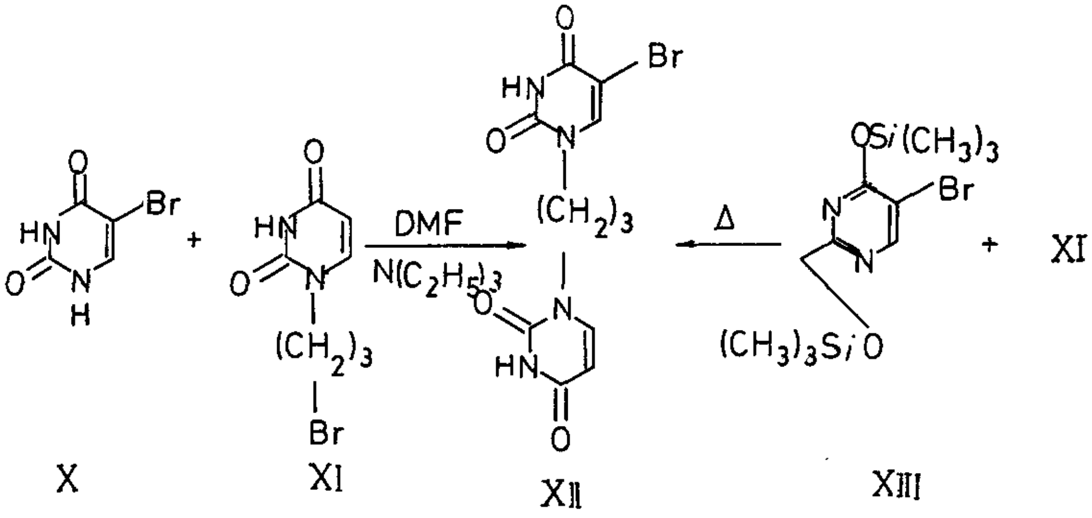
VIII, R= CH_3
IX, R= C_2H_5

Scheme 2

Products VIII and IX were obtained without isolation of any intermediates as VII. Reaction medium after completion of the bromination reaction is acidic and heating catalyses the HOBr elimination reaction from intermediates VII converting it to products VIII and IX, respectively.

The method of the direct bromination of 1,1′-trimethylenebis-uracil (II) does not permit to introduce selectively bromine atom only into 5-position of this system for synthesis of 1,1′-trimethylenebis-(5-monobromo)- uracil (XII) . This compound was synthesised by alkylation of 5-bromouracil (X) with 1-(3-bromopropyl)-uracil

(XI) in DMF solution and in the presence of $N(C_2H_5)_3$ as a base as well as by alkylation of 2.4-bis-O-trimethylsilyl-derivative (XIII) of 5-bromouracil with XI Scheme 3 .



Scheme 3

It is interesting to note that alkylation of uracil itself with 1-(3-bromopropyl)-5-bromouracil under the same conditions does not give XII. Instead of XII we have isolated the substance whose structure is now under elucidation. Identification of reaction products was based on elemental analyses as well as UV, NMR, MS and IR data. The compounds obtained are listed in Table 1, and their UV data in Table 2.

From UV data it is evident that introduction of the bromine atom into 5-position of 1,1′-trimethylenebis-uracil system causes significant changes of λ_{max} position of long wavelength absorption band and its intensity, for which $\pi - \pi^*$ character was described[20], in comparison to 1,1′-trimethylenebis-uracil. In all studied cases we observed bathochromic shift of this band. For example, 5BrUra(1 $(CH_2)_3$ 1)Ura in comparison to Ura(1 $(CH_2)_3$ 1)Ura has λ_{max} bathochromically shifted by 6.5 nm. Introduction of the second bromine atom into this system additionally shifts bathochromically this band by 11 nm. The positions of λ_{max} for synthesised bromo-bis-pyrimidines contrast also with 1,1′-trimethylenebis-alkyluracils (λ_{max} from 266.5 - 272 nm)[1,19], and 1.3′-trimethylenebis-alkyluracils (λ_{max} from 265 - 270 nm)[21].

Described bathochromic effect is in accord with 5-bromouracil itself (in comparison to uracil) for which this change of λ_{max} position was explained in terms of electronegativity (and electron affinity) or "bulkines" (or van der Waals' radii) of halogeno substituent as well as by the mesomeric effect of bromine coupled with π electrons of C5 $=$ C6 double bond of this molecule[20,22].

Compound[x]	Melting point °C	Analyses, %					
		C		H		N	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
5BrUra(1 (CH$_2$)$_3$ 1) Ura $C_{11}H_{11}N_4O_4Br$ m.w. 343.146	292	38.5	38.5	3.2	3.2	16.3	16.2
5BrUra(1 (CH$_2$)$_3$ 1) Thy $C_{12}H_{13}N_4O_4Br$ m.w. 357.166	284-285	40.4	40.1	3.7	3.5	15.7	15.6
5BrUra(1 (CH$_2$)$_3$ 1) 5EtUra $C_{13}H_{15}N_4O_4Br$ m.w. 371.196	248-249	42.1	41.0	4.1	4.0	15.1	15.8
5BrUra(1 (CH$_2$)$_3$ 1) 5BrUra $C_{11}H_{10}N_4O_4Br_2$ m.w. 422.05	308-311	31.3	31.4	2.4	2.5	13.3	13.1

x/ Symbols used in accord with suggestions of Cohn et al.[18].

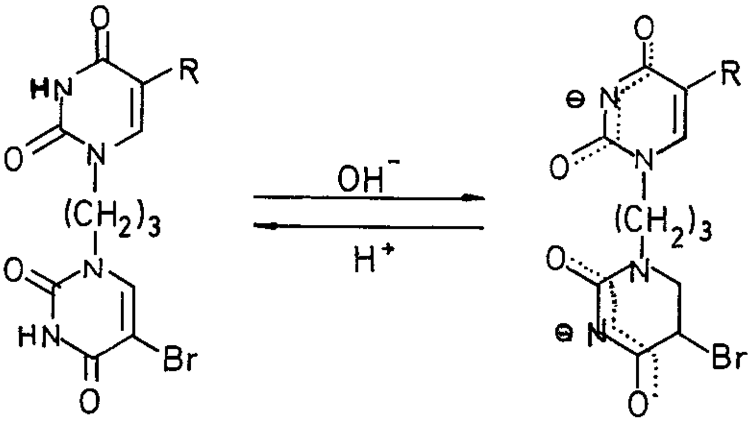
Table 1. The analytical data of bromine derivatives of 1,1'-trimethylenebis-uracils.

For neutral molecule of 5BrUra(1 (CH$_2$)$_3$ 1)5BrUra it is possible that some steric effects also exist beside electronic ones because its λ_{max} of long wavelength absorption band increases strongly in comparison to 5BrUra(1 (CH$_2$)$_3$ 1) Ura what correlates well with the stacking interaction conception for polymethylenebis-uracils[1].

Bathochromic shift of the long wavelength absorption band of 1,1'-trimethylene-bis-(5-bromo)-uracils is decreased for their dissociated forms (see Table 2, 0.01N NaOH solutions). This effect was also observed by Shugar et al.[22] for

1-methyl-5-bromouracil.

Broad and asymmetric as well as of reduced intensity the long wavelength absorption band for basic solutions of 1,1′-trimethylenebis-(5-bromo)-uracils may consist of an equilibrium mixture of two ionized forms of these compounds (Scheme 4).



Scheme 4

Formerly this was observed, for example, by Shugar et al.[22] and by Fox et al.[23] for 5-bromouracil, by Wierzchowski et al.[24] for uracil, thymine, 5-fluorouracil and other 2.4-diketopyrimidines, and by us for 1.3′-trimethylenebis-alkyluracils[21]. However, Nakanishi et al.[25] were first who formulated explanation for singly ionized form of uracil in aqueous medium.

NMR data of the compounds obtained are presented in Table 3.

It is well known that a strongly electronegative atom or group attached to or near a magnetic nucleus has the effect of deshielding the nucleus[27].

Taking into account the NMR results of Kokko et al.[28,29] for 5-bromouracil itself, we have also observed for 1,1′-trimethylenebis-uracils that the introduction of an electronegative substituent as bromine atom into 5-position of these systems shifts 6C-H proton resonance into low-field region of NMR spectra of 0.25 - 0.35 ppm. This deshielding effect is probably transferred through the bond system Br-C(5) = C(6)-H.

From Table 3 it is evident also that for other protons of pyrimidine systems under consideration, the deshielding effect of bromine is less significant (0.03 - 0.11 ppm) than for 6C-H protons (see TFA-d_1 solutions), which in this case probably is transferred through space.

Compound	H_2O				0.01N HCl				0.01N NaOH			
	λmax	ε max	λ min	ε min	λmax	ε max	λ min	ε min	λmax	ε max	λmin	ε min
5BrUra(1(CH$_2$)$_3$ 1)Ura	273	15580	239	4040	273	15870	239	4250	271	12090	247	7000
5BrUra(1(CH$_2$)$_3$ 1)Thy	276	16450	241	3840	276	15930	241	4090	274	12100	249	6330
5BrUra(1(CH$_2$)$_3$ 1)5EtUra	276	14950	241	3590	276	15120	241	3780	275	11660	249	6260
5BrUra(1(CH$_2$)$_3$ 1)5BrUra	284	16820	246	3300	284	16660	246	3050	281	12470	253	5350
Ura(1(CH$_2$)$_3$ 1)Ura x)	266,5	19730	232	2970								
Ura(1(CH$_2$)$_3$ 1)Thy xx)	268	18630	235	3660								
Ura(1(CH$_2$)$_3$ 1)5EtUra xx)	268	18440	235	3650								

x), xx) Included for comparison from [1] and [19], respectively.

Table 2. Ultraviolet spectral data of bromine derivatives of 1,1′-trimethylenebis-uracils.

Compound	Solvent	5BrUra moiety		Ura moiety			Thy moiety			5EtUra moiety				N-CH$_2$-	C-CH$_2$-C
		6C-H	3N-H	5C-H	6C-H	3N-H	5C-CH$_3$	6C-H	3N-H	5C-C-CH$_3$	5C-CH$_2$-C	6C-H	3N-H		
5BrUra(1(CH$_2$)$_3$ 1)Ura	DMSO-d$_6$	8.23 (s,1)	11.72 (s,1)	5.54 (d,1 J=8)	7.64 (d,1 J=8)	11.24 (s,1)								3.69 (t,4, J=7)	1.99-1.83 (m,2)
5BrUra(1(CH$_2$)$_3$ 1)Thy	TFA-d$_1$	8.01 (s,1)					2.07 (s,3)	7.52 (s,1)						4.00-4.20 (m,4)	2.87-2.40 (m,2)
	DMSO-d$_6$	8.23 (s,1)	11.72 (s,1)				1.75 (s,3)	7.88 (s,1)	11.20 (s,1)					3.67 (m,4)	1.99-1.83 (m,2)
5BrUra(1(CH$_2$)$_3$ 1)5EtUra	TFA-d$_1$	8.02 (s,1)								1.23 (t,3, J=7,5)	2.64-2.28 (m,2)	7.48 (s,1)		4.09 (m,4)	2.64-2.28 (m,2)
	DMSO-d$_6$	8.22 (s,1)	11.72 (s,1)							1.03 (t,3, J=7,3)	2.21 (q,2, J=6)	7.45 (s,1)	11.18 (s,1)	3.68 (m,4)	1.99-1.83 (m,2)
5BrUra(1(CH$_2$)$_3$ 1)5BrUra	DMSO-d$_6$	8.23 (s,2)	11.74 (s,2)											3.70 (t,4, J=6,5)	2.09-1.84 (m,2)
Ura(1(CH$_2$)$_3$ 1)Ura x)	TFA-d$_1$			6.20 (d,2, J=8)	7.76 (d,2, J=8)									4.11 (t,4, J=7)	2.35 (m,2, J=7)
Ura(1(CH$_2$)$_3$ 1)Thy xx)	TFA-d$_1$			6.17 (d,1, J=8)	7.70 (d,1, J=8)		2.03 (s,3)	7.47 (s,1)						4.2-3.8 (m,4)	2.30 (m,2)
Ura(1(CH$_2$)$_3$ 1)5EtUra xx)	TFA-d$_1$			6.16 (d,1, J=8)	7.67 (d,1, J=8)					1.20 (t,3)	2.7 2.0 m,2	7.37 (s,1)		4.2-3.8 (m,4)	2.7-2.0 (m,2)

x),xx) Included for comparison from [1] and [26] , respectively

Table 3. NMR spectral data of bromine derivatives of 1,1'-trimethylenebis-uracils.

EXPERIMENTAL

The melting points are uncorrected and were measured on a Kofler apparatus. UV spectra were recorded on Specord UV/VIS (C. Zeiss, Jena), NMR spectra on JEOL FX 90Q 90MHz in TFA-d_1 and DMSO-d_6 solutions using TMS as internal reference, MS spectra on JEOL JMS-D-100, and IR spectra on Perkin-Elmer 580 in KBr pellets. Elemental analyses were carried out on Elemental Analyzer Perkin-Elmer 240. 5-Bromouracil was synthesized according to Hilbert at al.[30], and Wang[14], 2.4-bis-O-trimethylsilyl-5-bromouracil by method of Wittenburg[31], and 1,1′-trimethylenebis-alkyluracils by method devised formerly[9].

Synthesis of 1-(3-bromopropyl)-5-bromouracil

2.4-Bis-O-trimethylsilyl-5-bromouracil (0.3 mole) was mixed with dry 1.3-dibromopropan (4.8 mole) and then the mixture was kept at 110° for 2 - 3 hours. After cooling to the room temperature the mixture was poured into distilled water (1l) and then extracted with chloroform (4 x 500 ml). Chloroform extracts were dried (sodium sulphate) and then concentrated in vacuum. Obtained solution was mixed with hexan (1l) and separated solid material was collected and after recrystallization from isopropyl alcohol pure product was obtained (76% yield, m.p. 186 - 187°C).

UV: H_2O (λ_{max} = 281 nm, ε_{max} = 8220)
0.01N HCl (λ_{max} = 284nm, ε_{max} = 9200)
0.01N NaOH(λ_{max} = 280nm, ε_{max} = 6640)

NMR ($CDCl_3$, δ scale, ppm) : 7.59 (s,1,6C-H); 3.94 (t,2,J=6,7Hz,N-CH_2-);
3.45 (t,2,J=6.1Hz; N-C-C-CH_2-);
2.43-2.13 (m,2,C-CH_2-C)

MS; 25 eV; m/z (% rel. int.): 314 (40; M+2); 312 (76, M); 310 (40; M-2)

Anal. Calcd. for $C_7H_8N_2O_2Br_2$; C, 26.9; H, 2.6; N, 9.0
Found: C, 27.0; H, 2.5; N, 8.9.

Synthesis of 5BrUra(1$(CH_2)_3$ 1)Ura

Method A.

To the solution of 5-bromouracil (0.1909 g; 1 mmole) in 10 ml of dry DMF, triethylamine (1.089 g; 10.8 mmole) was added and mechanically mixed. After mixing for 20 min 1-(3-bromopropyl)-uracil[1] (0.2564 g; 1.1 mmole) was added to the homogeneous solution. After 72 hours the solvent was distilled off under reduced pressure and the residue was mixed with 15 ml of chloroform/methanol 1:1 (vol./vol.). The separated solid material was collected and recrystallization from water gave pure product (45% yield).

Method B.

To 2.4-bis-O-trimethylsilyl-5-bromouracil (2.9 g; 8.6 mmole) was added 1-(3-bromopropyl)-uracil[1] (0.43 g; 1.8 mmole) and then the mixture was kept at 110 - - 120°C for 5 hours. Unreacted 2.4-bis-O-trimethylsilyl-5-bromouracil was distilled off and the residue was mixed with acetic anhydride (3ml) and heated under reflux. Unsoluble substance was separated by filtration and washed with acetic anhydride and Et_2O and dried. The second portion of product was obtained from acetic anhydride solution by column chromatography on silica gel. After recrystallization from water of both portion of substance pure product was obtained (12% yield).

Synthesis of 5BrUra(1 $(CH_2)_3$ 1) Thy

Ura(1 $(CH_2)_3$ 1) Thy (0.1 g; 0.36 mmole) was suspended in 3 ml of destilled water and then 0.04 ml (0.78 mmole) of bromine was added and the mixture was mixing until substrate was dissolved. The solution was heated at 80°C for 10 min. and then concentrated in vacuum. To the oil residue 3 ml of EtOH was added. Separated solid material was collected, washed with EtOH and dried. Recrystallization from water gave pure substance (60% yield).

Synthesis of 5BrUra(1 $(CH_2)_3$ 1) 5EtUra

This compound was synthesized by analogy to 5BrUra(1 $(CH_2)_3$ 1) Thy in 52% yield.

Synthesis of 5BrUra(1 $(CH_2)_3$ 1) 5BrUra

Method A.

To 4.2 g (0.012 mole) of 2.4-bis-O-trimethylsilyl-5-bromouracil was added 1 g (0.0032 mole) of 1-(3-bromopropyl)-5-bromouracil. The mixture was then kept at 110°C for 3 hours. Unreacted 2.4-bis-O-trimethylsilyl-5-bromouracil was evaporated in vacuum and the residue was mixed with $CHCl_3$ (10 ml). Solid residue was in turn dissolved in DMF and product was separated after mixing DMF solution with Et_2O (10% yield).

Increase of reaction temperature to 150°C did not improve the yield of product.

Method B.

This substance was also synthesized by analogy to 5BrUra(1$(CH_2)_3$ 1)Thy in 85% yield.

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